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CONTENTS

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A GRAPHIC METHOD FOR RECORDING OXYGEN CONSUMPTION

By R. R. Hannon, M. D. and R. S. Lyman, M. D.

(From the Chemical Division of the Medical Clinic of the Johns Hopkins Hospital)

The purpose of this paper is to present briefly a graphic method of determining basal metabolism, which has recently been developed and used in the routine basal metabolism determinations of this hospital.

Several graphic methods of recording oxygen consumption have been devised. The method described by August Krogh [1] can be readily used with certain types of American apparatus. With his article in mind, together with the distinct need of simplifying the technic and reducing errors in our readings on the "Metabolor," which was obtained by the Chemical Division for the routine basal metabolism determinations of the hospital, a graphic recording device has been adapted to this form of apparatus. The same principle has been adapted to the Benedict Apparatus and the method has been reported in detail by Paul Roth.[2]

With the Metabolor as manufactured, as well as with some other forms of apparatus designed to measure oxygen absorption volumetrically, the determinations depend upon an initial reading made while the scale of gas volume is in motion and then upon a second reading at the end of the test-period. Although it does not require any special skill to read the scale, it was found that the two consecutive test-periods too often failed to check each other within the 5% which is considered the outer limit of necessary accuracy for the apparatus. Furthermore, the ideal patient in basal condition,—that is, adequately rested, lying motionless and relaxed and having been without food for 14-16 hours,—should breathe with absolute regularity, should strike the same position of the chest wall after each expiration and should absorb oxygen at exactly the same rate throughout the test. But the patients who come for observation are not always perfect in this respect, especially those who come to the hospital for only one morning through the dispensaries. And it has often seemed that in just those cases in which basal metabolic reports are most wanted the patients persistently show irregularities in breathing;

even though they have been without food and though they really try to lie still and co-operate. In such cases, reading the volume of air at the end of just two isolated expirations may give results so divergent that satisfactory checks cannot be obtained. For this reason one of us (R. R. H.) has constructed and added to our original apparatus a lever-arm and pointer which will give a graph of the respiration on a smoked-drum. In this way one obtains a continuous record of what goes on throughout the test. The data can then be drawn either from the whole picture or from any selected part, and also the whole process is preserved in permanent form. One can refer to it at any time and can satisfactorily compare successive tests which may be made on the same patient. The tidal air is noted, as well as the respiratory rate and regularity of breathing, in addition to the rate and evenness of oxygen absorption in each determination. With that before one it is possible to tell at a glance how much credence should be put in the figures calculated from a given record as the metabolic rate. Some results in which there have been uniform respiratory movements and good checks on repeated periods present no question as to their accuracy, but others are seen to be obviously unreliable, although repeated periods may by chance give readings which are not far apart. From such irregular records exact figures in reporting the metabolic rate are not warranted.

Our hope was not only to reject tests because of their unreliability, but also to salvage what might be otherwise valueless. In the case of those subjects with irregular breathing, who are available for only one morning and also in the case of those few patients who never do learn to breathe evenly through either a mouthpiece or a mask, the graphic picture often allows an interpretation which may be only approximate, but which should give in condensed form more trustworthy information about the true basal rate of oxygen absorption than can be obtained from two direct readings on the apparatus, even when close observation of the patient and notes about his condition are made. On such a graph one can usually interpolate a line which gives the best average of oxygen consumption, or one can select parts of the graph in which there is a steady drop in the air in the apparatus and project a line joining the peaks of those expiratory summits which are regular. From such projected lines estimations of the basal metabolism can be made and may serve as a more or less reliable approximation, depending upon the extent of the irregularities which present themselves.

The apparatus which has been used for this work is the "Metabolor," built by the Toledo Technical Appliance Co., Toledo, Ohio. It is one of several types of closed-circuit apparatus. The metabolor has a wedge-shaped air-chamber, somewhat similar to that of Krogh, which is floated in water. The spirometer carries a scale graduating the volume of gas at 25 c.c. intervals. These calibrations have been carefully checked by one of us (R. R. H.). A smoked drum is moved up against the pointer of the lever arm. Then, the metabolor-drum being held still at each successive liter mark indicated by the scale, the smoked drum is revolved once in each case, calibrating the latter at liter intervals, from 0 to 7 liters. Before starting a metabolism test, we draw into the air-chamber 2 liters of room air and then fill up to the 6- or 7-liter mark with oxygen from a tank set into the stand. That mixture of oxygen and room air is carried by a motor, placed outside of the air-chamber, through soda lime to remove CO_2; it then goes through a tube to the patient, whose expirations return via another tube to the metabolor-drum, from which we started to trace the circuit. Figure 1 shows the portable stand with metabolor, smoked drum and timer in place. One should make sure that all joints are tight,—which is easily tested by closing the circuit, weighing the drum, starting the motor and noting whether or not the gas volume remains constant,—and that the soda lime is not saturated with CO_2,—which is determined by bubbling the contents of the air-chamber after a metabolic test through $Ba(OH)_2$. Under those circumstances, any drop in the volume of gas in the metabolor-drum, while the patient is in the circuit, represents the absorption of that much oxygen by the patient, provided, again, that there are no leaks around the mouthpiece or nose-clip. The procedure of getting a record from a patient is simple and short. After breathing has become regular with the mouthpiece and nose-clip in place, the patient is brought into the circuit and the apparatus is allowed to run until the pointer recording on the smoked drum shows at least 2 liters of oxygen to have been absorbed. After an interval, the same process is then repeated to furnish a check. After each test the smoked drums can be immediately put on the pegs arranged for them on the stand until the tests for the morning are finished, when calculations can be made. First, we correct the gas volume for temperature and pressure and then turn attention to the graph. When the patient breathes out, his expired air goes into the metabolor and the pointer rises. Accordingly, the peaks of the curves occur at the end of expiration. In a satisfactory graph a straight line can be drawn which is just tangent to each expiration, provided that the smoked drum revolves at a constant speed. The projection of the intersections made by that line and the two adjoining horizontal liter-calibrations on to a line marked at 5-second intervals by the timer gives the length of time which it has taken the patient to absorb one liter of oxygen. This is shown in Figure 2. One then refers to a table for the factor by which this figure is multiplied in order to get the number of liters of oxygen consumed per hour. Benedict, Emmes, Roth and Smith [3] found the average R. Q. for 88 normal men and 66 normal women to be .82. Assuming that quotient, one multiplies the O_2 consumption per hour by the factor, which gives the heat

equivalent of one liter of oxygen. Having thus arrived at the heat production, reference to DuBois' chart [4] shows the surface area calculated from the height and weight. Division of the heat production expressed in calories per hour by the surface area gives the calories per hour per square meter of body surface. A table constructed by Aub and DuBois [5] shows the normal calories per square meter per hour for different sexes and ages. Comparison of the subject's heat production per unit of body surface with the average normal values obtained by Aub and DuBois enables one to determine the percentage variation of the metabolism from the average normal. The use of 4-place logarithms makes the calculation very simple. A sample form-sheet which is used, together with the data obtained in one of the routine tests, is shown in Table 1. After obtaining the necessary data at the time

TABLE I.

BASAL METABOLISM

Name Mooney Evans Date Jan. 21, 1923 Ward H.P.P.C. (Relf)
Diagnosis Normal man
Age 29 Sex M Height 164 cm Weight 62.8 kg Temp Surface area 1.68 M²
Mask on 1. About 1½ min. Valve open 1.
2. " 1 " 2.

Gas temp start 22.3 23.7 — Barometric reading 760.2
Gas temp end 23.5 25.0 — Barom temp 25.5
Average gas temp. 21.9 24.3 23.6 — Corrected Barom et Aq.V.P. 24.7
Pulse 1. 56 Resp. 1.
2. 56 2.

A.1. From 6 liters O₂ to 5 liters O₂ 48.9 Excellent
2. " 5 " " " 4 " " — (Leak; 44.1 projected)
3. " " " " " " "
B.1. " 7 " " " 6 " " 49.5 Good
2. " 6 " " " 5 " " 48.9 "
3. " 5 " " " 4 " " 48.5 "
4 | 195.8
48.9

Log. Barom. (Corrected) 735.5 8666
Log. Reduc. Av. Gas Temp. 23.6° 0831
Log. time factor. 48.9 5-min. interval 1680
Ht. value l. O₂ at R.Q. .83 6819
CAL. PER HOUR 63.0 7996
Surface area 1.68 7747
CAL PER HR PER SQ. M. 37.5 5743
" " " " " "(norm) 39.5 4034
% AVERAGE NORM. 95.00 9777
BASAL METABOLIC RATE —5

Log. Cal per hr. 7996
Log 24 38021
CAL PER DIEM 1513 1798

The form-sheet used in the routine determinations of basal metabolism.

the patient is going through the test (the underlined topics on the form-sheet) and after the graphic record is ruled off, it takes only from four to ten minutes, according to the number of irregularities presented, to write down the proper logarithm or cologarithm and make the single addition of six factors, the antilogarithm of which gives the final result.

The accuracy of the results obtained from this graphic method has been checked in three or four ways. In the first place, simultaneous direct readings were made on the metabolor-scale in seventeen cases. Of those, there were only eight in which the direct readings came within 5% of each other. That is, there were only eight cases in which direct readings appeared accurate enough to serve as checks at all. A summary of these cases is given in Table 2. Another standard of one's results is the comparison of successive readings on patients who have not received any treatment which would alter their meta-

TABLE II.

Comparison of Graphic Determinations with simultaneous direct readings on Metabolor.

Name	Date	Av. Graph. Reading	Direct Readings
Mrs. W.	Sept. 30, 1922	—13	—10 —9
Mrs. V.	Oct. 18th	+33	+36 +35
Miss McC.	Oct. 8th	—8	—5 —4
Mrs. P.	Oct. 8th	—5	—7 —4
Miss McE.	Oct. 9th	+2	+5 +8
Mrs. McG.	Oct. 12th	—2	—2 —6
Miss P.	Oct. 14th	—11	—11 —9
Mrs. R.	Oct. 18th	—6	—5 —2

Comparison of graphic determinations with simultaneous direct readings showing good agreement.

TABLE III.

REPEATED METABOLIC DETERMINATIONS			
M. E.— ..... (Normal)	Nov. 6th Nov. 13th	—12 —9	
M. H.— ..... ("Invalid")	Oct. 31st Nov. 17th	+6 +7	
J. M. W.— ..... (Depression)	Oct. 24th Nov. 3rd	—2 —5	
G. L.— ..... (Leukemia)	Oct. 21st Nov. 3rd Nov. 16th	+92 +59 +53	(poor record) (poor record) (poor record)
C. B.— ..... (Hyperthyroid before operation)	Nov. 8th Nov. 24th	+35 +36	
Mrs. K.— ..... (Hyperthyroid before operation)	Oct. 25th Nov. 6th Nov. 25th	+60 +38 +39	
Mrs. V.— ..... (Hyperthyroid)	Oct. 18th Nov. 4th Nov. 25th	+33 +36 +33	

Repeated determinations of basal metabolic rate on patients who have not undergone any active treatment which could affect the metabolic rate. The first readings on G.L. and Mrs. K. were made before the patients had been subject to prolonged rest.

bolic rate. We have a few cases of more or less value on that score, given in Table 3. Some patients have been run on both the metabolor and on other types of apparatus, as shown in Table 4. The CO_2 method mentioned

TABLE IV.
Comparison of Reports on Different Types of Apparatus.

Name	Metabolor	Tissot	CO_2
M. E.— ..... (Normal)	—5 (1-21-23)	—2.5 (1-21-23)	
F. S.— ..... (Tbc. kidney)	—10 (1-18-23)	—9 (1-18-23)	
E. W. R.— .... (CNS lues)	—9 (1-20-23)	—4 (1-20-23)	
C. B.— ..... (Hyperthyroid post-operative)	+26 (1-8-23)	+31 (1-8-23)	+29 (1-2-23)
J. J.— ..... (Hyperthyroid before operat.)	+42 (1-5-23)	+47 (1-4-23)	(50 ?)
W. A.— ..... (Diabetic)	+2 (11-9-22)	—4 (11-10-22)	
G. H.— ..... (Diabetic)	—3 (10-11-22)	—1 (11-19-22)	
I. P.— ..... (Diabetic)	—3 (11-15-22)	—3 (12-20-22)	
R. W.— ..... (Diabetic)	—21 (11-10-22)	—17 (12-27-22)	
F. H.— ..... (Leukemia)	+41 (12-2-22)		+44.6 (12-29-22)
C. H.— ..... (Hypothyroid)	—8 (12-1-22)		—10.5 (12-21-22)
R. J.— ..... (Hysterical hyperpnea)	+22 & +31 (11-21-22) poor record +19 (12-9-22)		+13.25 (12-8-22)
T. K.— ..... (Hyperthyroid)	+29 (12-5-22)		+35 (12-14-22) +29.9 (12-29-22)

Comparison of determinations made on the Metabolor by the graphic method agree satisfactorily with those made on other types of apparatus.

was worked up by Dr. John T. King, Jr.,[6] who has allowed us to use his apparatus and figures in this connection.

The most interesting part of the study of our graphs has been the detection of errors which may occur in the manipulation of an apparatus which measures oxygen volumetrically. To be sure, either the failure of consecutive periods to check, or the close observation of the patient alone would almost invariably disclose some inaccuracy in results without the graphic record. But, by means of a graph, the exact cause of trouble is usually so evident that it could not escape anyone's notice and occasionally one can correct for it without sacrifice of accuracy. Some examples of records obtained from patients during the last few months appear with comments on their main features.

SUMMARY

A method of graphic measurement of oxygen consumption is described which is simple and short enough for routine clinical use, similar to that devised by Krogh.

In using spirometers we have found the graphic method of recording oxygen consumption to be of distinct value in interpreting our results because:

1. It gives a permanent record of respiration throughout the test.

2. Errors of technique are usually self-evident.

3. Fractional parts of the graph can often be used when the record as a whole proves unreliable.

4. It shows at a glance whether figures which are calculated from it should be taken as accurate, approximate, or totally valueless.

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LEGENDS

Fig. 1.—Metabolor mounted on portable stand with timer, smoked-drum and mouth-piece in place.

Fig. 2.—Graph showing absorption of one liter of oxygen.

Fig. 3.—Satisfactory record.

Fig. 4.—Too irregular for accurate reading.

Fig. 5.—A leak around mouthpiece or nose-clip on inspiration.

Fig. 6.—The nose-clip became loose. A projected line allows a reading for that period which checks fairly well with the other readings.

Fig. 7.—The two periods fail to check, their slopes not being parallel. The patient was not strictly "basal" in the lower case.

Fig. 8.—The soda lime was saturated with CO_2, as was proved after the test by bubbling the remaining air in the spirometer through Ba(OH)2. The graph shows rapid breathing with increasing excursion of respiratory movements.

Fig. 9.—Hyperpnea—post-encephalitis.

Fig. 10.—Cheyne-Stokes breathing.

Fig. 11.—Regular irregularity allowing an approximate reading. Direct readings on the spirometer scale would be even more unreliable.

Fig. 12.—This patient was sent to the metabolic department without comment about her condition. The graph showed readings which did not check. The lowest metabolic rate which could be calculated was +132. It was then found that the patient had undergone an operation for empyema and had Dakin drainage at the time of the metabolic test, which accounted for the results obtained.

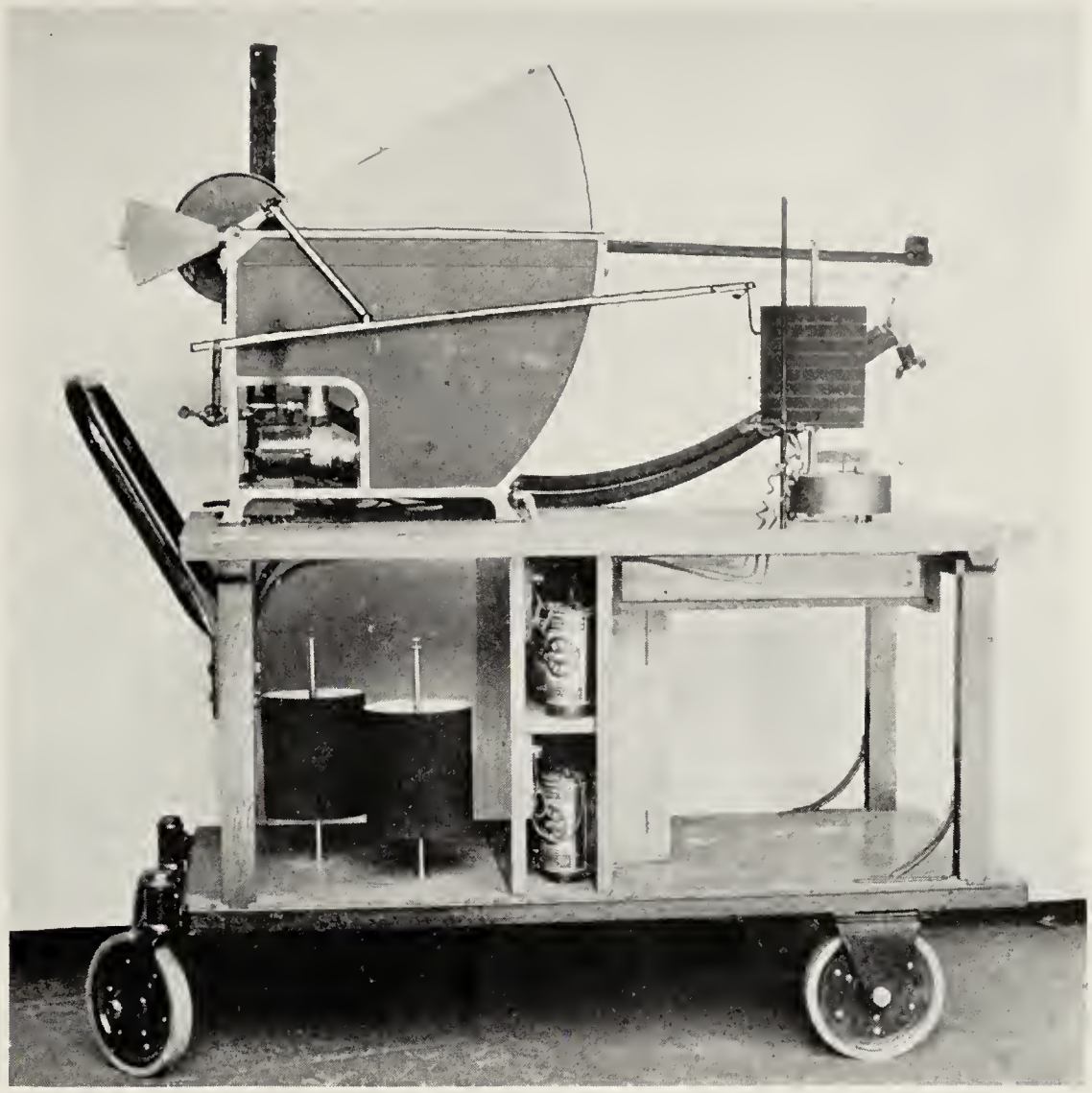


Fig. 1.

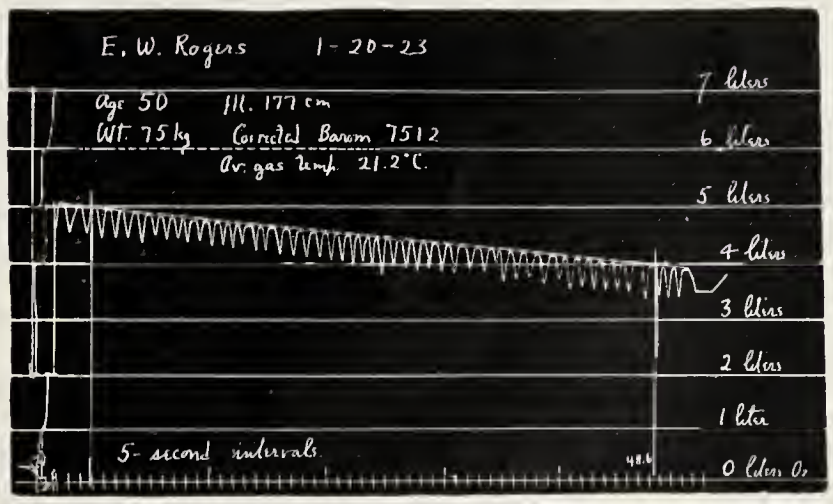


Fig. 2.

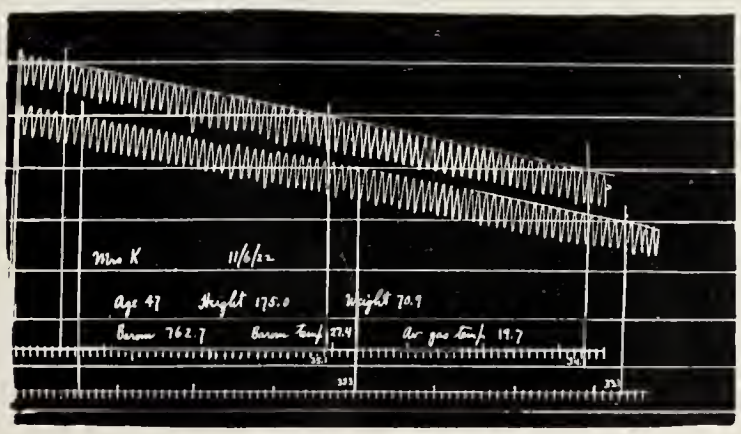


Fig. 3.

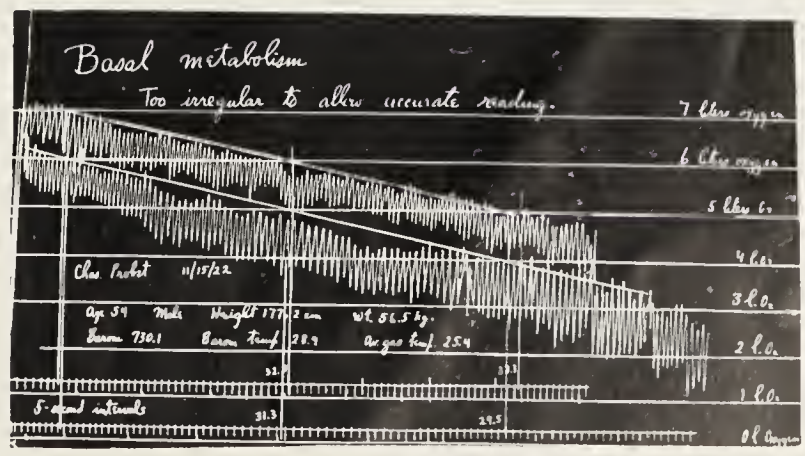


Fig. 4.

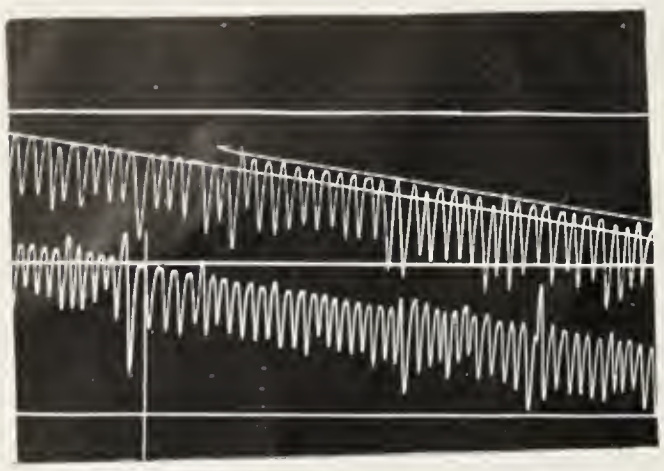


Fig. 5.

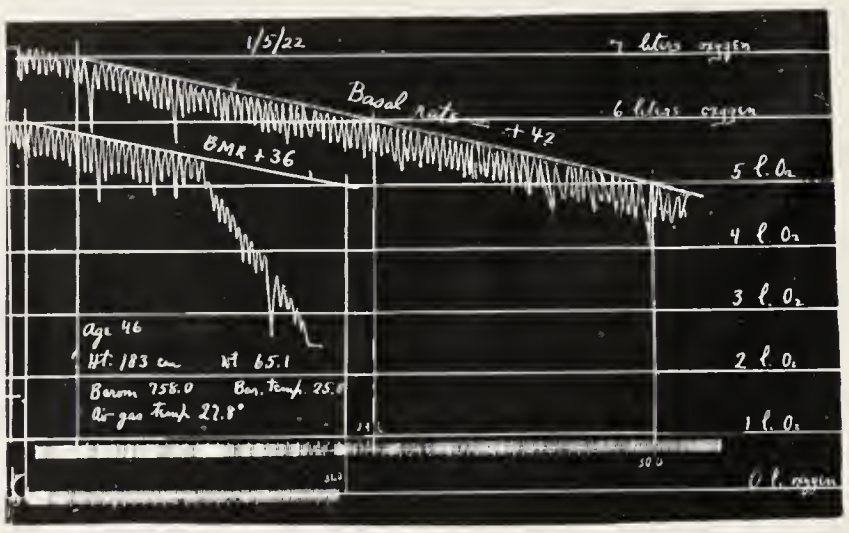
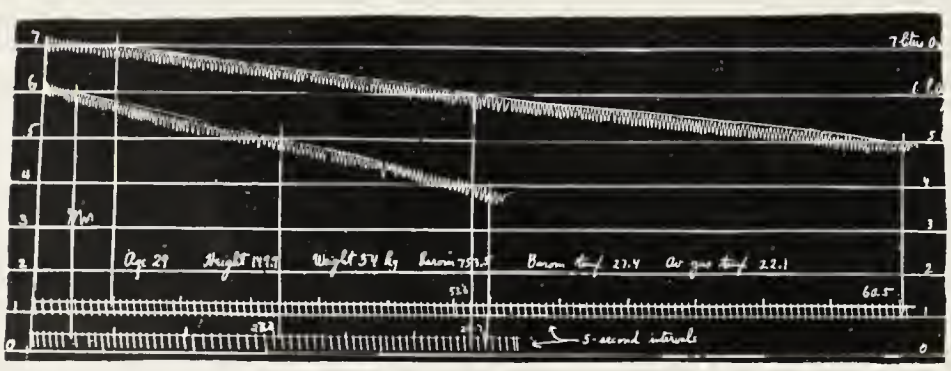


Fig. 6.



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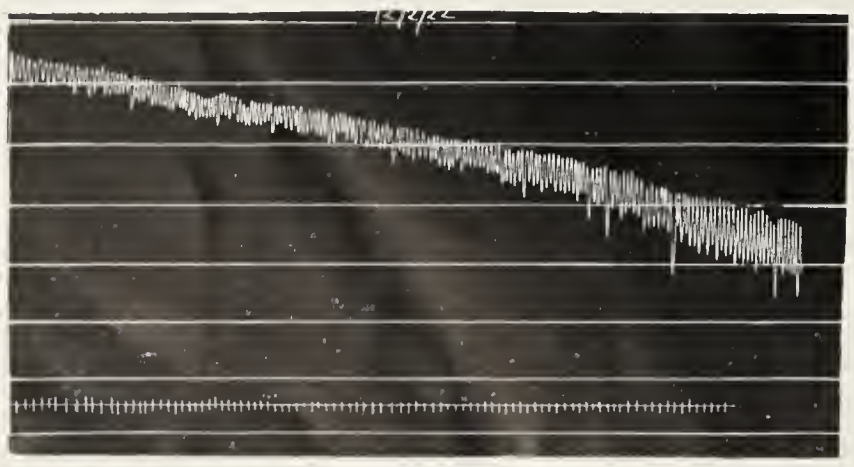

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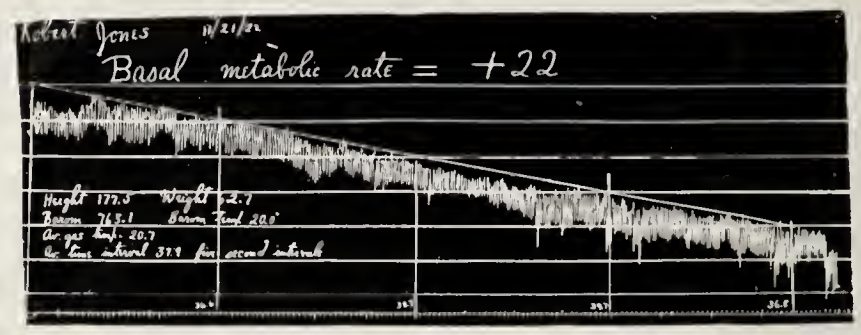

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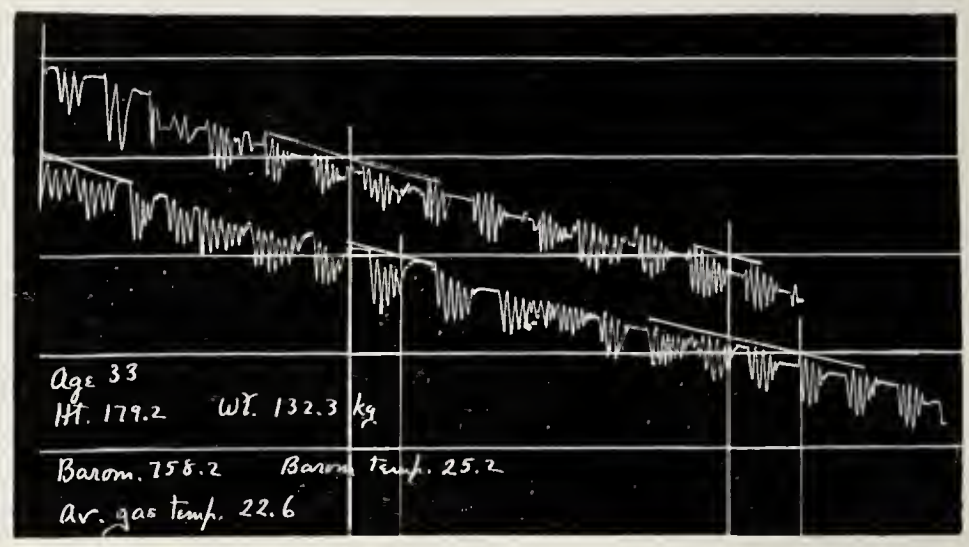

Fig. 10.

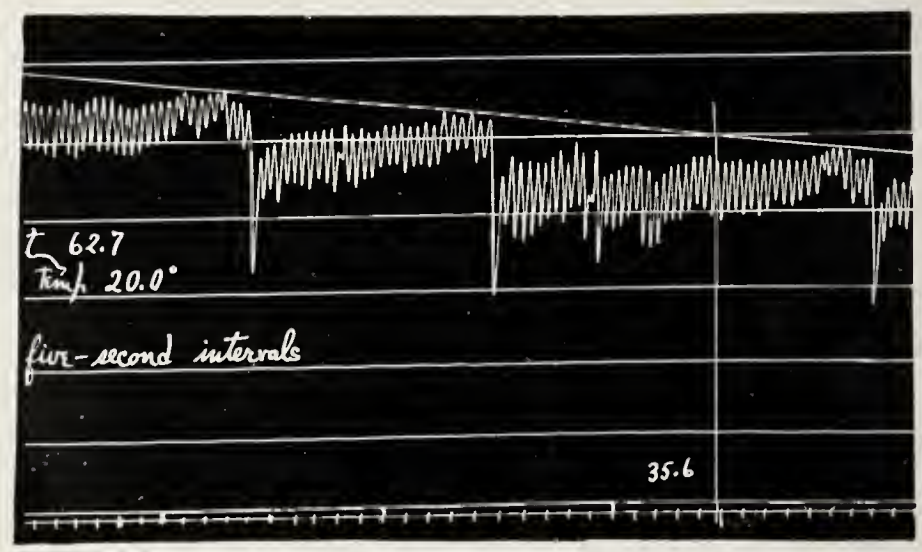

Fig. 11.

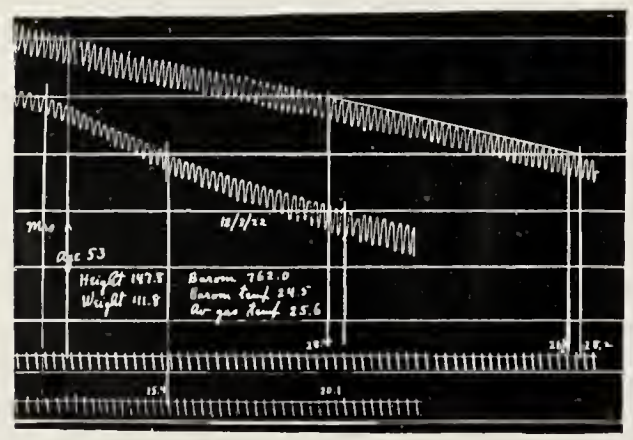

Fig. 12.

THE SPACE-COMPENSATING FUNCTION OF THE CEREBROSPINAL FLUID—ITS CONNECTION WITH CEREBRAL LESIONS IN EPILEPSY

By WALTER E. DANDY, M. D.

(From the Department of Surgery, the Johns Hopkins University and Hospital)

It is the generally accepted belief that the chief function of cerebrospinal fluid is to protect the brain and spinal cord from the ordinary and extraordinary shocks to which these important structures are subjected. The existence of great waterbeds around the most important parts of the nervous system—the medulla, spinal cord, pons and midbrain—would seem *primâ facie* to furnish sufficient proof of this protective function. Moreover, the prominent bony irregularities along the base of the human skull would appear to require a buffer against constantly recurring shocks. It is difficult to see how even the ordinary movements of the spinal column could be tolerated without damage to the spinal cord except through the protection afforded by the beds of cerebrospinal fluid.

The ability of waterbeds to protect their enclosed structures must lie in the ready displacement of fluid, for, being almost totally incompressible, water cannot absorb shocks as can an elastic body. The effects of a localized force are diminished by a loss of fluid at the pole of impact and by transmission of radial waves of fluid to more distant parts. The amount of injury which results locally and at a distance will, therefore, be dependent upon the severity of the blow and the amount of fluid at the site of the blow. A severe impact produces such a rapid radiation of fluid waves that an explosive effect results. By a series of ingenious experiments, Sir Victor Horsley [1] has shown that the amount of destruction of brain tissue, perpendicular to the axis of flight of the modern high velocity bullet, is proportionate to the content of water along its passage, and is due to the incompressibility of fluid. The velocity and cross-section of the bullet also are contributing factors to the degree of this tangential damage.

The other functions of the great cerebrospinal circulatory system have been but vaguely understood because of the great defects in our knowledge of its anatomy and pathology. Although claims of chemical activity for the cerebrospinal fluid have been made, they have not been substantiated.

The function of cerebrospinal fluid is also a mechanical one, and in this paper I shall discuss its adaptability to acute and chronic disturbances within the cranio-vertebral chamber.

[1] Horsley, Sir Victor: Remarks on gunshot wounds of the head. Brit. Med. Jour., 1915, I, 321.

The cranio-vertebral chamber in the adult is a fixed cavity, i. e., there can be no contraction and no expansion of its bony walls except under extraordinary circumstances such as erosion of the cranium or vertebrae. For the present, our attention may be restricted to the intracranial lesions, since those occurring within the vertebral chamber, though obeying the same fundamental mechanical laws, are smaller and of purely local importance. Nearly all intracranial lesions in greater or less degree require or produce changes in space, either transiently or permanently, and these space alterations must be met by the intracranial contents—the brain tissue, the blood, and the cerebrospinal fluid.

From a mechanical viewpoint, we may look upon lesions as (1) space-occupying and (2) destructive. In the first group are tumors, abscesses, tubercles, gummata, haemorrhages, hydrocephalus, meningitis, post-traumatic and post-febrile edema, reactions around foreign bodies, etc.; to the second group (i. e., the destructive) belong the late results of emboli, thrombi, infections, haemorrhages, trauma, etc.

A tumor's growth may be used as an example of the space-occupying lesions, for the mechanical adaptation to other lesions is similar. It is well-known that an intracranial new growth as large as one's fist is still compatible with life. But since none of the intracranial contents—brain tissue, blood and cerebrospinal fluid—are at all compressible, much of the extra room occupied by this foreign invader must be made up by a reduction in amount of these intracranial constituents. The existence of such a large tumor is possible only because of its slow growth, which permits the compensation to be gradually accomplished. A certain amount of room within the cranial chamber is obtained as the result of both a local and a more or less general anaemia of the brain. The proof of this statement is that subcortical brain tumors are detected by a pronounced local anaemia directly over the tumor; but, in addition, there is an anaemia of relatively less degree over more or less of the surface of the brain which is exposed at operation, this cerebral pallor becoming progressively less as the distance from the tumor increases.

Space is also obtained by the destruction of brain tissue. When a tumor does not block the cerebrospinal fluid channels and produce hydrocephalus, the destruction of brain tissue is principally contiguous to the

tumor; but when hydrocephalus has resulted, there is the added destruction due to the dilated ventricles. The extent of the loss in hydrocephalus varies with the number of ventricles blocked and whether the block is partial, or intermittent as with a ball-valve, or complete; but it is nearly always greater and more rapid than the localized loss around the tumor. Proof of the destruction of brain tissue over a tumor is easily shown by a softening of the brain detectable by touch and not infrequently, as in hydrocephalus, by the actual measurement of the thickness of the brain. At times in hydrocephalus, the entire brain tissue between ventricle and the meninges will have disappeared.

Probably a greater amount of room for the direct expansion of a tumor is obtained through the cerebrospinal spaces. This is easily demonstrable at the operating table and through cerebral pneumography, particularly ventriculography. Just as tumors reduce the blood vascular bed in the brain, so they reduce the spaces in the brain by forcing the fluid from them; (they may, however, as just noted, block the main channels and produce hydrocephalus). One of the characteristic operative findings over a subcortical cerebral tumor is the absence of cerebrospinal fluid and, consequently, the obliteration of the subarachnoid spaces, greater locally and less at a distance. In like manner, tumors of the posterior cranial fossa obliterate the cerebellar subarachnoid spaces and reduce the size of the cisterna magna. Ventriculography has shown in a graphic way how the ventricular cavities are used for gaining room. So important is this that every tumor large enough to give symptoms of intracranial pressure will reduce the size of the ventricular system. (It may also, of course, block the ventricles and produce hydrocephalus). These deformities have been so characteristic as to be nearly infallible indicators of the approximate or precise location of the tumor. The tumor always reduced the size of part or all of the contiguous ventricle and not infrequently of other ventricles. For example, a tumor in the left cerebral hemisphere may not only reduce the size of the left lateral ventricle, but it may compress the 3rd ventricle and part of the right lateral ventricle. In her desperate efforts to preserve life, nature has recourse to other methods to gain room. Of very considerable importance in tumors of the posterior cranial fossa is the utilization of the upper vertebral canal. In this effort, the cerebellar tonsils are forced through the foramen magnum, at times as far as the axis. This defence is precluded in supratentorial lesions by the intervention of the tentorium cerebelli. Although supratentorial pressure stretches the tentorium and reduces the size of the posterior cranial fossa, this membrane is so resistant that the cerebellum is rarely, if ever, pushed into the spinal canal by supratentorial tumors. A very limited amount of space may be gained by erosion of the skull. Tumors without hydrocephalus produce principally a local destruction over the tumor (at times the reverse may be true, i. e., there may be hypertrophy of bone over the growth). Infrequently this local loss of bone may be complete and a spontaneous decompression results. Hydrocephalus produces a more widespread atrophy of the inner table, the characteristic digital markings of convolutional atrophy, as shown in a roentgenogram.

But, important as are the cerebral cavities in space-occupying lesions of the brain, they seem even more necessary in destructive lesions. Although in the former the cerebral spaces yield but a part of the total space required, in the latter they appear to be called upon to make up for an overwhelming share of the loss of space.

Again, considering the cranio-vertebral chamber as a closed and fixed space, any loss of tissue must be made up by some other intracranial constituent. Although an increased intracranial pressure may be tolerated, a vacuum is inconceivable. One of the most frequent questions asked by onlookers after the removal of a brain tumor is: What becomes of the large space left by extirpation of the growth? One might also wonder what must result when a large cerebral artery becomes occluded, for the cerebral arteries are mostly end-arteries. Following deprivation of its only blood supply, the brain softens and gradually is absorbed. As this loss of cerebral tissue occurs, filling of its space is brought about by a corresponding enlargement of the cerebral spaces holding cerebrospinal fluid. Depending on the size and position of the cerebral defect, either the cerebral ventricles or the subarachnoid spaces or both will dilate to equalize the defect. If the lesion is near the surface, the subarachnoid spaces will be the chief means of compensation. If the defect is large and more deeply situated, the ventricle will form a pouch to fill it.* Very frequently both the ventricles and the subarachnoid spaces will be dilated in response to the loss of brain tissue. The end-results of emboli, thrombi, destructive effects of trauma, the space remaining after a haemorrhage has been absorbed, and various diffuse atrophies of the brain, occurring at all ages and as yet poorly understood, cause a loss of brain tissue which is replaced by dilatations of the fluid-containing spaces (Fig. 1).

Nature also fills cerebral defects in part or entirely, just as in defects elsewhere in the body, with fibrous tissue. Some cerebral defects contain but little gross fibrous tissue, others a great deal; some show a great dilatation of the fluid spaces and others very little; the exact proportion of these two processes doubtless depending on the character and extent of the intracranial lesion,

* Recently Dr. Elman and I have found (unpublished studies) that after excision of an area of cerebral cortex in animals, there is a compensatory enlargement of the lateral ventricle on the corresponding side, producing what might erroneously be interpreted as a unilateral hydrocephalus.

and on other factors not yet understood. The proof of these generalizations concerning the end-products of repair in intracranial defects is as follows: (1) The enlargement of the cerebral ventricles, principally the lateral ventricles, can easily be shown by ventriculograms. (2) The evidence of dilatation of the subarachnoid spaces, as well as fibrosis (or gliosis), will be discussed later in this paper.

So far, only conditions within the adult skull have been considered. Intracranial lesions of infancy and childhood are followed by similar changes, but there are differences in the method of adaptation on account of the plasticity of the skull prior to the union of the cranial sutures. In a general way (with exceptions, as in primary bony abnormalities) the skull conforms, where possible, to the intracranial contents. Increased intracranial pressure will cause separation of the cranial bones and an enlargement of the skull as far as possible. This is shown in tumors with or without hydrocephalus. When there has been destruction of brain tissue, the intracranial loss is compensated by a premature closure of the fontanelles. Contraction, from a head of normal size and shape to one which is smaller and of symmetrical shape, is not uncommon after destructive cerebral lesions in early infancy.

It would be a fruitless effort to attempt at this time an analysis of the greatly divergent views concerning the etiology of epilepsy. It is not because brains of epileptics do not show lesions, and not that these lesions have escaped detection, but they have not been consistently or regularly demonstrated. A gross defect involving a part or all of a hemisphere in one patient's brain may be matched by another with little if any gross deformity. Again, the numerous changes described in the brains of epileptics have been so varied in character and position and so inconstant as to appear rather as coincidental than as causative lesions. As a result of the inconsistent findings, it has come to be the belief of many that, aside from the relatively few instances with the Jacksonian "march," epilepsy is an idiopathic disease. Even the great cerebral deformities are not emphasized in the causation of epilepsy.

Although the convulsive seizures of epilepsy have many minor differences, they are all fundamentally similar. The various initial manifestations of the attacks—aurae—such as the sensory or motor Jacksonian "march," the unilateral motor or sensory onset without a "march," uncinate symptoms, visual disturbances, etc., are soon lost in the general convulsion. But these aurae, of whatever kind, have precisely the same localizing value as the "march," which led Hughlings Jackson to discover facts regarding cerebral function several years before the actual stimulation experiments of Fritsch and Hitzig and of Ferrier. All attacks cannot have precise aurae because of the large silent areas of the brain and because the area of the brain implicated is not always sharply defined.

There are all gradations between the attacks with sharp focal initial signs and symptoms of Jacksonian epilepsy, those with less well defined but still discernible unilateral signs, and those without unilateral manifestations. The less distinct the first sign, the more quickly the convulsion becomes general.

From a series of 75 brains of epileptics exposed at operation, exclusive of a large series of cases in which convulsions occurred in patients with brain tumors, abscesses, foreign bodies, etc., I wish to present evidence of the frequent presence of gross organic lesions in epilepsy. Through the courtesy of Dr. W. T. Shanahan, I have also had the opportunity of examining a collection of brains from the Craig Colony. They have greatly added to my studies. The results of the latter examinations will appear later.

Epilepsy is a symptom. Convulsions which cannot be distinguished from the so-called idiopathic form of the disease are found in a great variety of cerebral diseases. The so-called idiopathic form by its very name presupposes that there is no fundamental pathological change in the brain. I am of the opinion that in most instances it is possible either to demonstrate the presence of a cerebral lesion or some accompanying change which indicates that a lesion is present. I wish to discuss at the present time chiefly the gross changes that may be detected before or during the operation. The changes that one finds are: (1) dilatation of the ventricles, (2) abnormally shaped ventricles, (3) dilatation of the subarachnoid spaces, (4) softening of the brain (atrophy), (5) areas of increased density (fibrosis or gliosis), (6) changes in the meninges.

Ventricular changes are especially striking where the lesion is restricted to one hemisphere. Such lesions cause enlargement of part or all of one lateral ventricle. Very frequently in large unilateral cerebral atrophies the contralateral ventricle is also dilated. Any part of a lateral ventricle or an entire ventricle may enlarge to compensate for the loss of brain tissue (Figs. 2, 3, 4). When the lesion is bilateral and diffuse, the lateral ventricles are so nearly symmetrical that, unless the dilatation is extreme, it is impossible to say that the ventricles are not of a large but normal size. Lateral ventricles which we have no reason to assume to be abnormal may be three or four times the size of other ventricles, also presumably normal. Marked enlargement of part of a lateral ventricle is readily detected at operation. The cortex may have entirely disappeared in places, or it may be distinctly thinner and soft to the touch. All grades of asymmetrical ventricular dilatation are demonstrable by ventriculography and the location of the ventricle's enlargement is of course precisely located. An example of this kind was presented among the earliest ventriculo-

grams published by the author,[2] and since then many others have been demonstrated by this method, and many have been disclosed at operation. I have been particularly impressed with the high percentage of cases of asymmetrical enlargement of a lateral ventricle with localized cerebral atrophy from the series of brains in Dr. Shanahan's collection at Sonyea. These will be reported on at length in a subsequent communication. A large proportion of these patients had had a monoplegia, hemiplegia or diplegia.

Several deformed ventricles, both symmetrical and asymmetrical, have been demonstrated by ventriculography, and also from the pathological material at Craig Colony. Such distorted ventricles indicate congenitally malconstructed brains.

It is mainly at operation that dilatation of the subarachnoid spaces is demonstrable (Fig. 5). After death the fluid quickly disappears, and particularly after the brain has been hardened this indicator is to a large extent lost. When very marked, it can still be made out even after fixation, but there is never the striking appearance *post mortem* which is present in the brain at operation. One of the great advantages of inspecting the brain at operation is the comparison of its volume with that of the intracranial cavity. There is hardly anything more striking than the collections of fluid which cover the surface of the brain of the epileptic. They stand out almost as sharply as tumors on the surface of the brain. Instead of the normal convolutions separated by narrow sulci containing a small amount of clear fluid, one sees *accumulations of fluid completely covering areas of the brain* to such an extent that the underlying cortex and its vessels are entirely invisible. Whereas normally the pia-arachnoid is in close contact with the surface of the convolutions and dips down between them, in epileptics the subarachnoid spaces are dilated and fluid passes freely across the convolutions. Instead of lines of fluid, there are now pools which communicate freely with one another. This communication can be shown by injecting a dye into the subarachnoid space. From the point of injection, the dye can easily be pressed along for a considerable distance. This shows that these collections of fluid are merely dilatations of the subarachnoid space, and not cysts. The extent and distribution of the fluid is variable. It may be quite localized as over a post-traumatic area, or may cover a large part of the exposed hemisphere, and (as shown by necropsy material) most of the outer surface of both hemispheres may be covered.

Eliminating the focal lesions of known origin, such as healed infections, traumatic and vascular defects, etc., most of these brains show the maximum amount of fluid over the region of the motor cortex. There is less over the occipital and frontal lobes. The frontal lobe is usually implicated more than the occipital, the temporal scarcely at all. I have not found collections of fluid over the inferior surface of the temporal, frontal and occipital lobes. The reason for this fairly uniform distribution is not clear. *These collections of fluid indicate to my mind that there has been a loss of brain tissue, in amount at least equal to the quantity of fluid.* The size of such a cerebral defect is striking. The amount of fluid varies up to 100 c.c., or even much more. After release of the fluid by pricking and pressing it out, a cavity is seen between the brain and the dura, which varies in size with the extent of the cerebral lesion. There is no essential difference between the appearance of the brain surface from a case of Little's Disease and from one due to any other kind of lesion, except in degree and the distribution of the fluid. Although quite variable, the fluid covering the brain of a patient afflicted with Little's Disease is very extensive in depth and latitude.

The meninges are definitely changed over the defective portion of the brain. Both color and texture differ from the normal. The color varies from a semitransparent pink to an opaque white. The contrast with the normal delicate transparent pia-arachnoid is striking. Part of the opacity is due to the depth of the bed of fluid, for, after this has been released and the leptomeninges have settled upon the brain, the opacity may either be partially or entirely lost.

The pia-arachnoid is thicker and tougher than normal. This can be demonstrated by inserting a dural hook into the arachnoid and gently pulling. The membrane is often so strong that the entire brain can be moved by the pull, in marked contrast to the normal delicate membrane which tears so readily. The opaque white arachnoid is thicker than the more transparent type. The thickening is not always uniform, but may occur in patches which are much whiter and more opaque. Histological examination shows that the thickening is due to an increase of connective tissue.

After the cerebrospinal fluid over the affected area has been evacuated, the striking visual demonstration that the brain is abnormal is largely lost. At this stage, the appearance is more nearly comparable to the brain at necropsy. But abnormalities in the brain are still evident, though closer inspection is required. There is a definite depression where the fluid has been released. The convolutions are narrower and sharper, the sulci deeper and wider.

But palpation reveals a striking change of a different character in the brain. *The brain beneath the collection of fluid is softer than the contiguous normal brain.* When the gloved finger or brain spatula gently presses upon the normal brain, the indentation is slight because the resistance is that of a firm tissue, whereas, on palpation of this defective area, the finger or spatula sinks more deeply and the resistance imparted to the finger is

[2] Dandy, W. E.: Localization or elimination of cerebral tumors by ventriculography. Surg., Gyn. and Obst., April, 1920, p. 329.

less than in the normal brain. The transition between the normal and this diseased part of the brain may be gradual or quite sharp.

In this connection it should be stated again that softening of the brain tissue is by no means specific for any particular kind of lesion. It merely indicates that a lesion of the brain exists. This softened area may be associated with a small tumor, a cyst, an area of atrophy, or it may be and frequently is found with the so-called idiopathic epilepsy. It is often very difficult to be certain of the nature of a lesion more than a centimeter below the cortex. But there are differences between the softened brain over the larger subcortical tumors and purely atrophic brain lesions. In the former, the convolutions are wider and paler, the sulci are shallower, and the fluid over the surface is usually entirely squeezed out by the pressure.

In addition to the dilated ventricles, the collections of fluid, the cerebral atrophy and the areas of softening in the brain, there is at times evidence of fibrosis, either in or below the center. This may be detected at operation by a visible scar if superficially situated, or by the impact of a ventricular needle if subcortical. When a ventricular needle is passed gently through the cerebral tissue, the scar causes an increased resistance to the needle's passage. It may be so dense as to require considerable force before it can be penetrated. In 5 cases in which this has been demonstrated at operation, there has been a previous history of trauma, with its etiological relationship to the epilepsy uncertain though probable. In one instance, a dense scar was encountered in the wall of the ventricle and extended along most of the body of the ventricle. In the other cases, the scar was encountered at varying but lesser depths beneath the cortex, and in all but one of these the cortex appeared nearly normal. In one case, the scar occupied an extensive area in the hemisphere and extended into the wall of the body and descending horn of the lateral ventricle, producing incomplete strictures of both the body and descending horn. A partial extirpation of nearly all of this mass was found to be possible without injury to the motor or sensory tracts. In all save one of these cases with fibrosis, there was a marked atrophy of the brain with a bed of fluid over the cortex. It will depend upon the degree of cerebral atrophy or of fibrosis whether the brain is softer or firmer than normal to touch, and whether the passage of the ventricular needle is easier or more difficult.

Another gross finding, which has been noted a few times in the localized lesions, is the presence of a few drops of fluid which drip from the ventricular needle when withdrawn from the lesion, whereas in the normal brain no fluid can be obtained. This finding indicates that there are small pockets of fluid within the substance of the lesion.

The microscopic examination of material removed at operation is limited to three cases in which a localized lesion had been excised from silent areas and examined only with haemotoxylin and eosin staining. Two were from areas of softening without gross evidence of fibrosis, the third from a very dense fibrous growth. In all three the microscopic findings were essentially similar. The cortex seemed approximately normal, but beneath it there were collections of fibrous tissue, often in compact areas, and interspersed throughout the section were small cavities apparently containing fluid, but without an endothelial lining. The spaces doubtless account for the softening to touch as well as for the fluid obtained by puncture with the ventricular needle.

I do not by any means assume that a similar histological picture will be found in the brains of all epileptics, certainly it will not. I desire to place no emphasis upon the histological picture at this time, other than to point out that the softening of the brain may properly be considered a trustworthy indication of a subcortical lesion.

The increase of fluid over the brains of epileptics has long been recognized, both at necropsy and operation. As early as 1885, the meningeal changes were well described by the great English neurologist and student of epilepsy, Sir William Gowers.[3] But he considered the changes to be the product and in nowise the cause of the convulsions. The frequency of this finding is also recorded in the pathological reports of Prout and Pierce Clark (1904) [4] and of Shanahan (1911-1921) [5] from the extensive necropsy material at Craig Colony. Prout and Pierce Clark concurred in the view of Gowers that the fluid collections are merely the result of the attacks, and this, I believe, is the prevalent impression at the present time.

Alexander (1911) [6] was greatly impressed with these collections of fluid observed at operation and considered them to have a direct bearing on the cause of epilepsy. He particularly emphasized the greater prominence of these findings at operation than *post mortem,* when they might well be overlooked. He attempted to reproduce these subarachnoid collections in animals, but failed to produce epilepsy. For the cure of epilepsy, he suggested and carried out quite extensively an operation which he termed "fenestrations of the dura." But both his experimental and curative efforts failed, and with these failures his claims for the relationship of the fluid collections with epilepsy were discredited. He mistook the effect (the fluid) for the cause (the cerebral atrophy).

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[3] Gowers, W. R: Epilepsy and other chronic convulsive diseases. New York, 1885.

[4] Prout and Pierce Clark: *in* Spratling: Epilepsy and its treatment. New York, 1904.

[5] Shanahan, W. T.: Annual reports of Craig Colony, 1911-1921.

[6] Alexander, Wm.: The surgical treatment of some forms of epilepsy. Lancet, Sept. 30, 1911, p. 932.

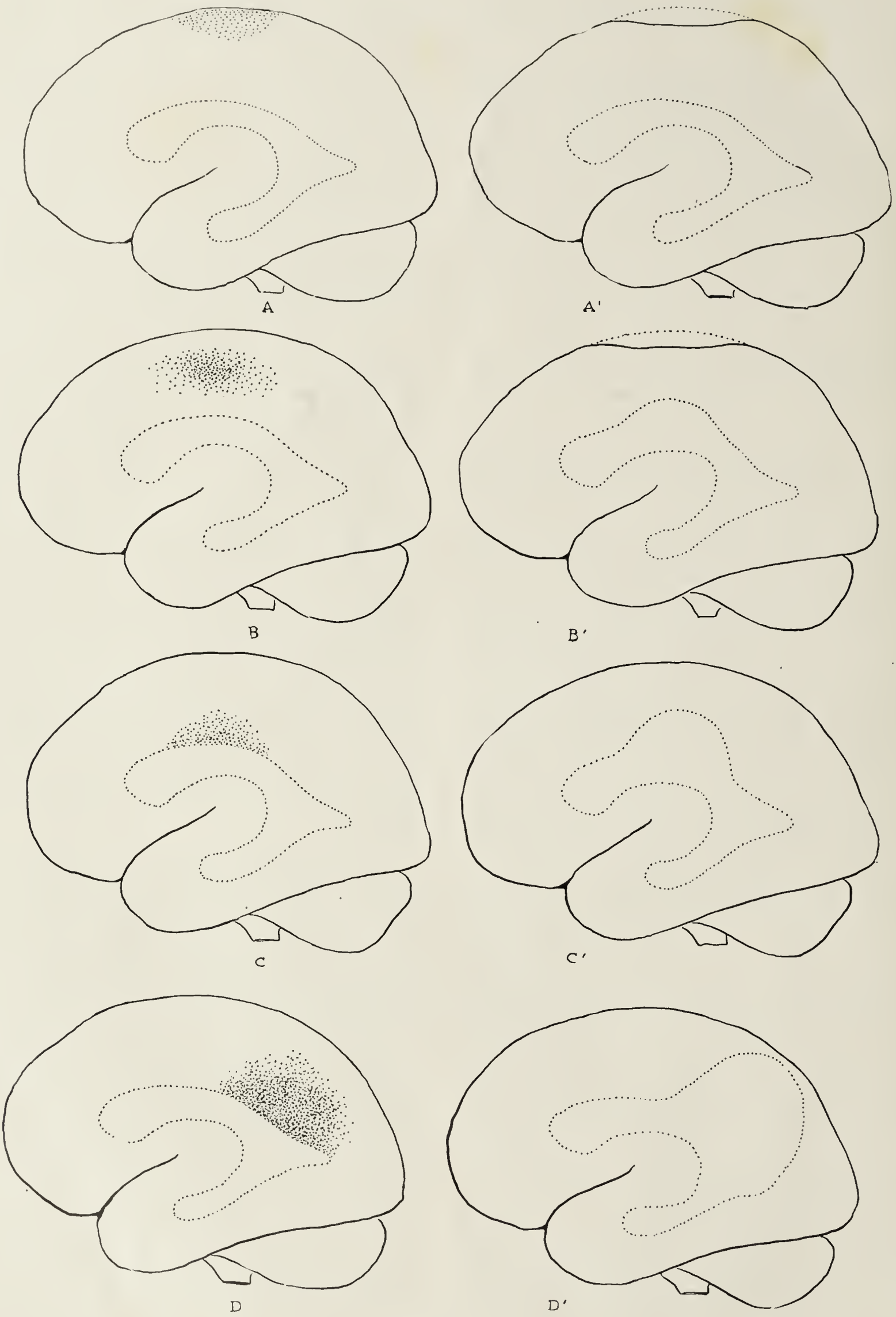

Fig. 1.

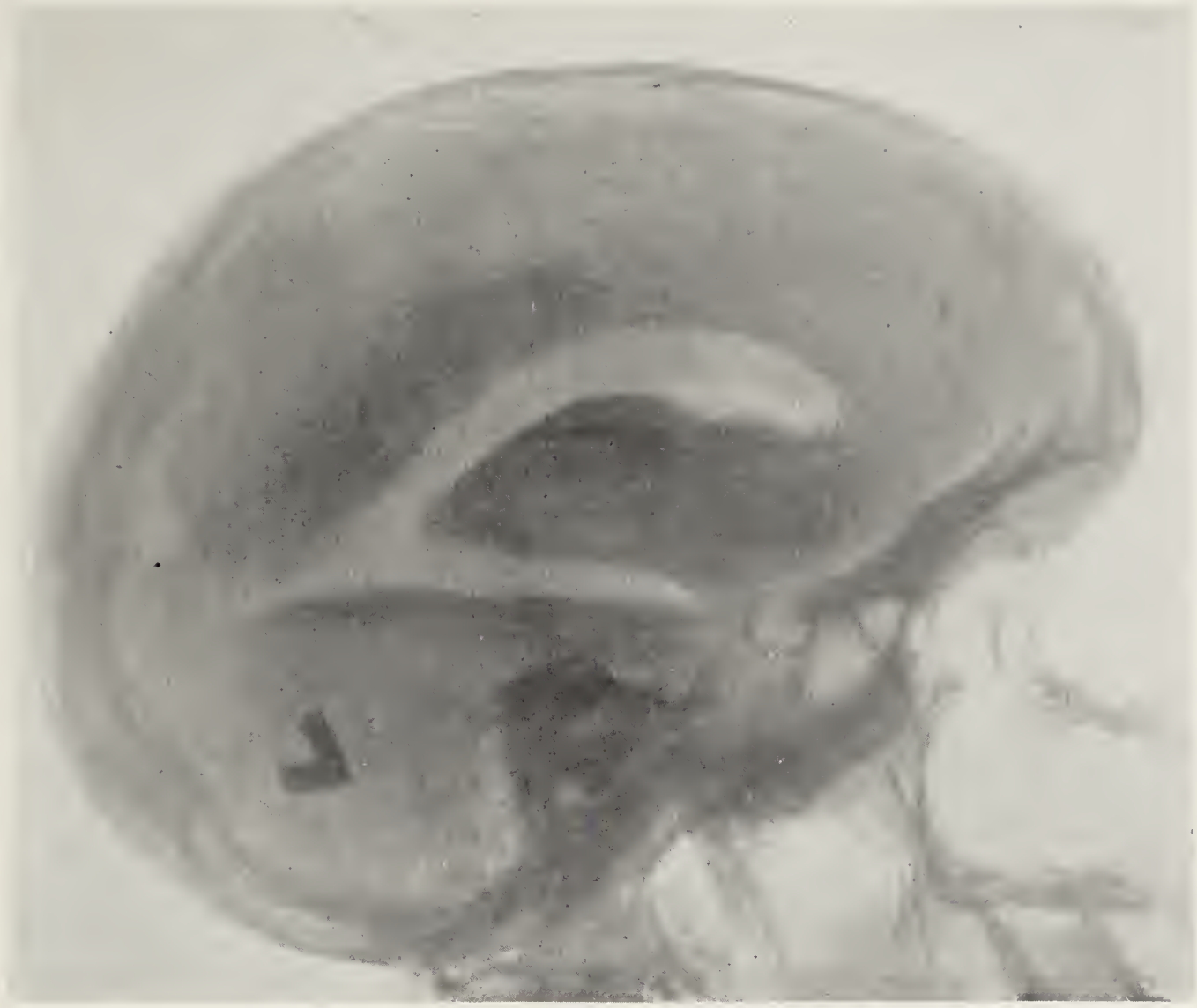

Fig. 2.—Left lateral ventriculogram of normal size in patient with epilepsy. (Slightly retouched to preserve detail.)

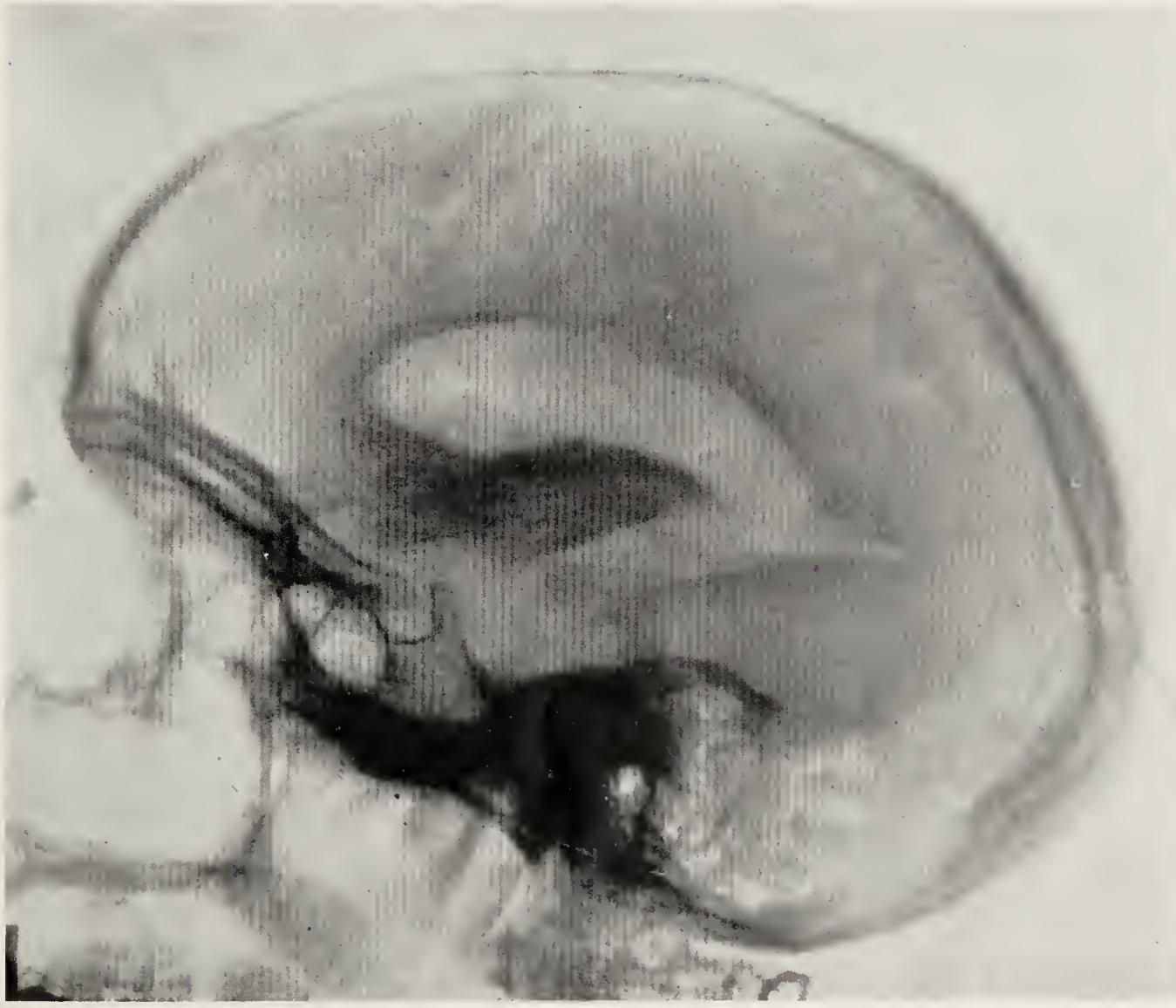

Fig. 3.—Right lateral ventriculogram showing fusiform dilatation of ventricle on side of lesion (mate of Fig. 2) in patient with epilepsy. (An extreme example of unilateral ventricular enlargement was shown in Surg. Gyn. & Obst., April, 1920.)

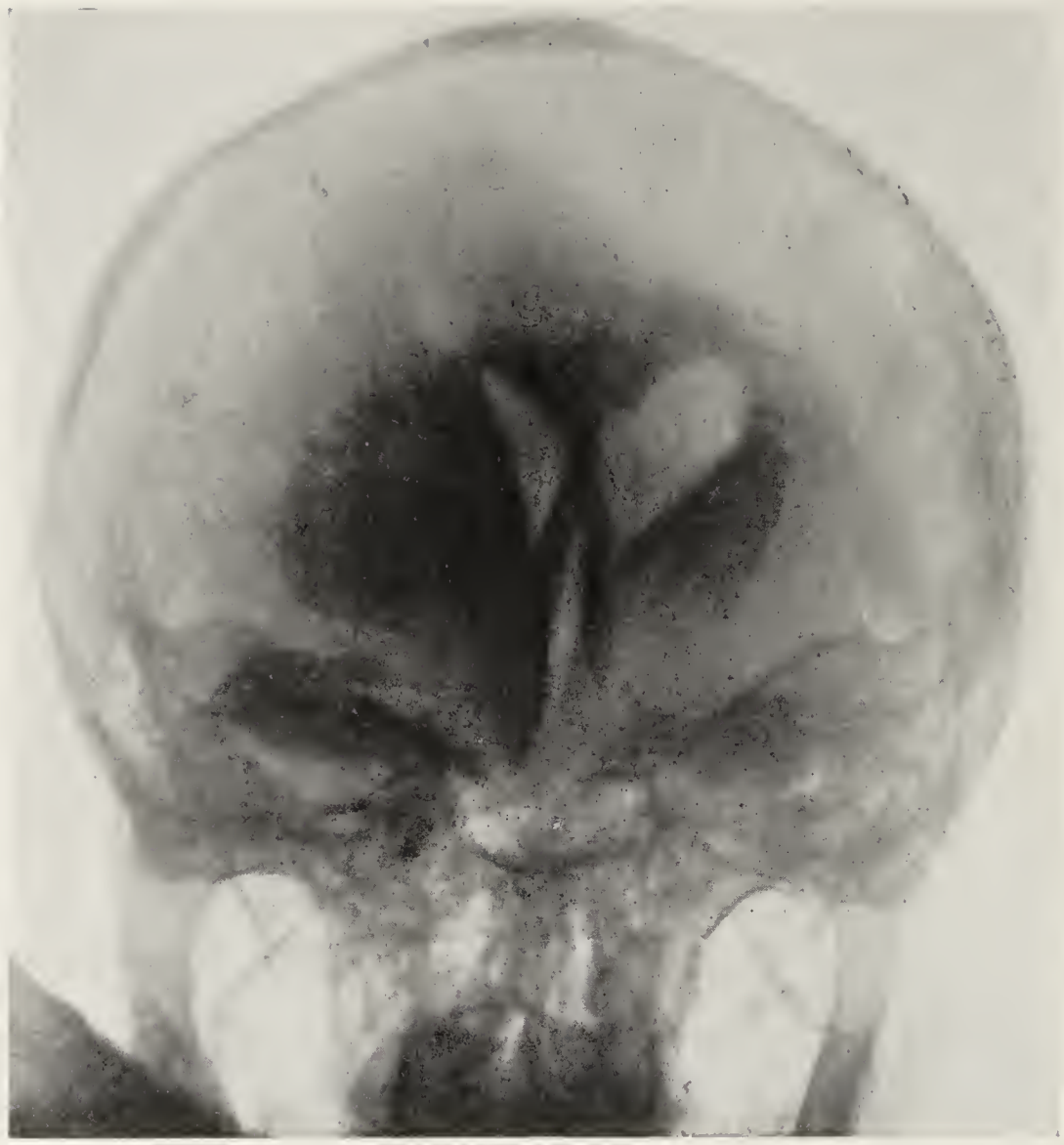

Fig. 4.—Anteroposterior ventriculogram, from same patient (Figs. 2 and 3) showing asymmetry of ventricles, the larger being on the side of the lesion.

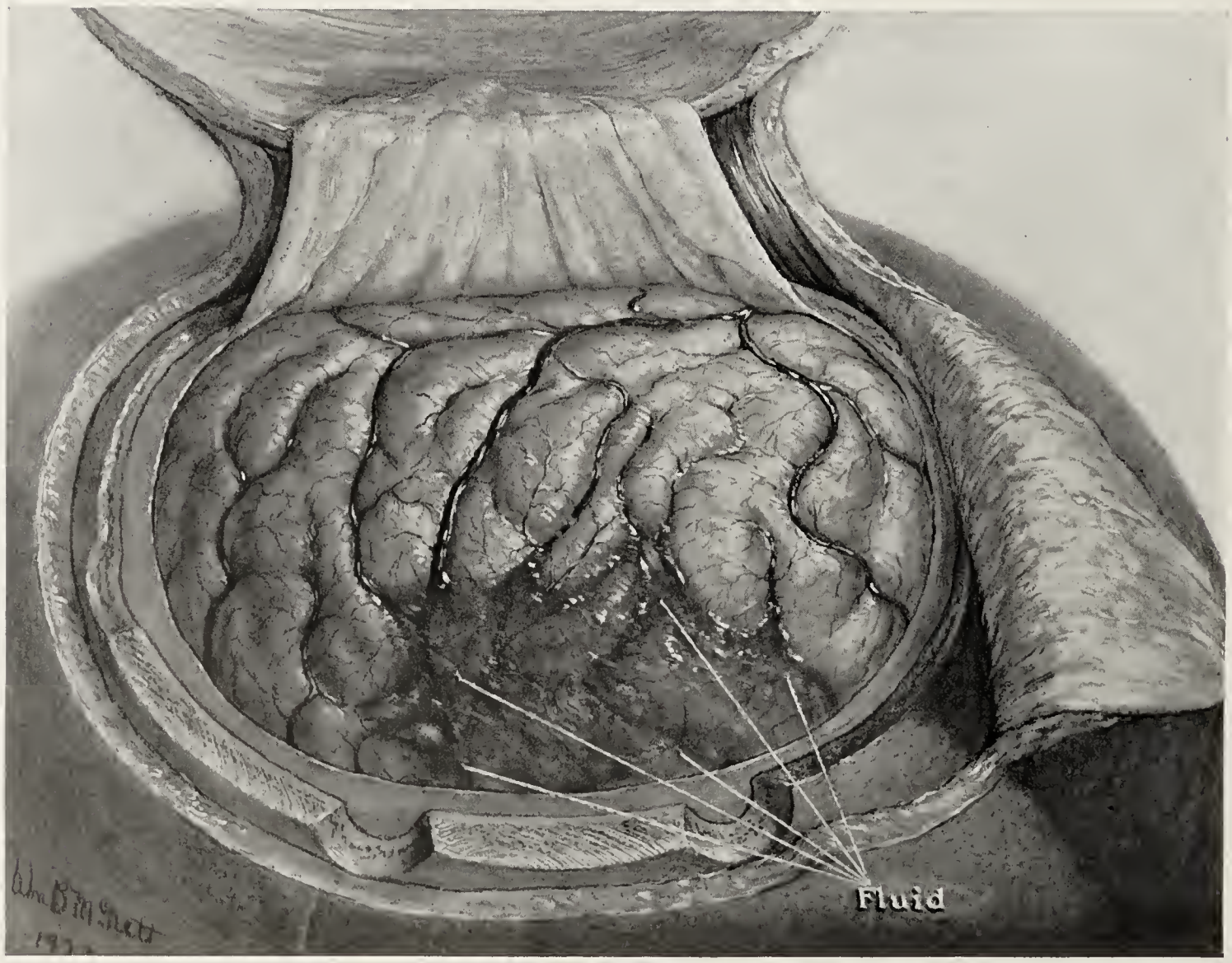

Fig. 5.—Drawing from brain at operation to show a bed of fluid over a localized area of cerebral softening. In other cases of a more diffuse character, the pools are distributed over more or less of the hemisphere's surface. In extreme grades, practically the entire hemisphere may be obscured.

But these changes in the meninges and the enlargement of the subarachnoid spaces cannot be dismissed with the statement that they are a result of the convulsions, for many of the most severe cases of epilepsy (the congenital type) do not have an increase of fluid. If convulsions should in any conceivable manner produce such intracranial alterations, it would be difficult to explain why the fluid collections are in many instances so strictly localized and why the process is not universal.

The occurrence of a layer of gliosis under the pia has been described by Féré,[7] Bleuher,[8] Alzheimer[9] and others.

The findings described are by no means found in all cases of epilepsy, but they are, I believe, to be expected in one form or another in cases that are acquired after birth. Congenital defects of the brain may or may not be associated with plainly marked external signs. The soft plastic skull of intra-uterine life may more readily be moulded to the defective brain. But congenitally malconstructed brains are frequently well-shaped and symmetrical, i. e., there may be no gross defects to fill with fluid or alter the shape of the skull, so that the meninges and the subarachnoid spaces will appear quite normal.

In the course of operations for intracranial lesions other than epilepsy, areas of atrophied brain presumably similar to those described in epilepsy have been found in a few instances. These were nearly all chance findings, and are not related to the lesion for which the operation was performed. It could hardly be doubted that were a series of brains examined at operation in individuals apparently normal, a not inconsiderable percentage would show this lesion. The lesions surely can be present without epilepsy. The relationship of cerebral lesions to epilepsy may be compared to that of cerebral tumors to epilepsy. Only a certain proportion of brain tumors cause epilepsy. Of two tumors in different individuals, apparently similarly situated and of the same type, one will cause epilesy and the other will not. There are reasons which we cannot yet determine, but which are responsible for the seeming lack of uniformity in the causation of symptoms in many different diseases. There are also surely great differences in the susceptibility of individuals to convulsions and doubtless there are differences in the intrinsic character of the lesions, although with our coarse methods of examination they may look alike.

[7] Féré, Ch.: Revue de méd., 1895, XVI, 115.
[8] Bleuher, E.: Münch. med. Wchnschr., 1895, XXXIII, 789.
[9] Alzheimer: Monatschr. f. Psychiatrie u. Neurol., 1898, IV.

SUMMARY

In a series of operations for the relief of epilepsy, a number of changes have been found with considerable uniformity. These are: (1) dilatation of ventricles, (2) collections of fluid on the surface of the brain, (3) pockets of fluid in the brain substance, (4) softening of the brain in association with these collections of fluid, (5) areas of induration in the brain (fibrosis or gliosis), (6) changes in the meninges, and (7) congenital malformations. It is believed that these are evidences of actual cerebral lesions (end-products of repair), and the frequency of the findings leads to the conclusion that there is a pathological basis for so-called idiopathic epilepsy in a large proportion of the cases. Confirmation of this view is obtained by means of ventriculography, which in a certain proportion of cases shows acquired or congenital distortion of the ventricles. A more detailed study of the lesions will be made at some future time.

PROPHYLACTIC VACCINATION AGAINST ACUTE TONSILLITIS*

By ARTHUR L. BLOOMFIELD and AUGUSTUS R. FELTY

(From the Biological Division of the Medical Clinic, Johns Hopkins University and Hospital)

In the course of a bacteriological and epidemiological study of acute tonsillitis[1] it was found that carriage of beta-hemolytic streptococci in the tonsils seemed to prevent attacks of this disease. In brief, among forty-nine carriers one case occurred, an incidence of 2.5%, whereas among fifty non-carriers there were twenty-eight or 56% of cases of acute tonsillitis during a period of six months. It appeared, furthermore, that resistance was directly related to the continued presence of the streptococcus in the carrier and that a high degree of susceptibility was rapidly re-established after the carrier state was terminated. These observations seemed to furnish a rational basis for prophylactic vaccination.

Material.—Ninety young women—students in the Johns Hopkins Hospital Training School for Nurses—were voluntary subjects of the experiment. Control cultures for the presence of beta-hemolytic streptococci in the throat and tonsils had previously been made and the appearance of the tonsils had been noted. The entire group was under close supervision during the period of the experiment, which ran from January 1, 1923, to May 1, 1923.

Vaccine.—A polyvalent vaccine was prepared from twenty-one strains of beta-hemolytic streptococcus recently isolated from cases of acute follicular tonsillitis. The organisms were grown for twenty-four hours on human blood agar slants. The growth was scraped off, washed with salt solution, centrifuged, re-suspended, killed at 65° C. for one hour, diluted, counted, tested for

* This is the seventh of a series of papers on streptococcus infection, with special reference to Acute Tonsillitis.

sterility, and preserved with 0.3% phenol. Before administration to the experimental group the vaccine was titrated by preliminary tests on a few normal people. An amount which contained about 100,000,000 bacteria produced an area of slight redness, soreness and swelling of the arm which varied in size up to 10 cm. in diameter and persisted for one to three days. There was usually slight general malaise.

Vaccination was commenced in the group on December 27th, 1922. Three doses of 75M, 100M, and 125M were given at intervals of one week by subcutaneous injection into the arm. Mild reactions as described above followed practically all of the injections—in no case was the subject incapacitated.

Of the ninety members of the group thirty-three were carriers. Seventeen were vaccinated and sixteen were used as controls. There were fifty-seven non-carriers and of these eighteen received the vaccine.

Results.—Before reporting the results it may be said that the vaccine was subjected to a severe test. During the months of January, February, and March, 1923, there was an epidemic of influenza, which, as is well known, predisposes to streptococcus infection. Further evidence of a general increase in the activity of the hemolytic streptococcus was made apparent by the development of acute streptococcus infections of the lymphoid tissue of the throat (equivalent to tonsillitis) in people whose tonsils had been removed—a type of infection which had not been present in the group during the previous months. Thus, among seventy-seven tonsillectomized students no infection occurred during October, November and December, 1922, although tonsillitis was already prevalent, whereas in the same group six cases developed during January, February and March, 1923.

DIAGRAM I.

Results of Vaccination Against Tonsillitis.

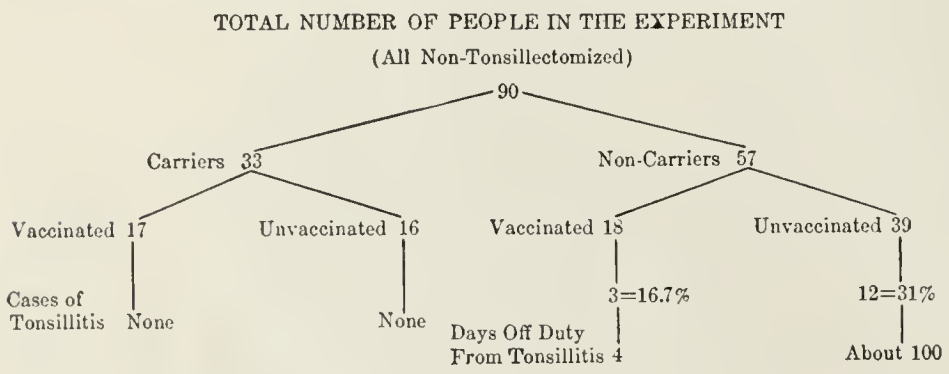

TABLE I.

Summary of Diagram I.

Carrier	Vaccination	Number	Cases of Tonsillitis
+	+	17	None
+	0	16	None
0	+	18	3=16.7%
0	0	39	12=31.0%

The results of the experiment are summarized in Diagram I and Table I. No case of tonsillitis occurred in the group of carriers whether vaccinated or unvaccinated, and this was in accord with our previous observations. In the group of non-carriers, on the other hand, there were fifteen persons who developed the disease, eight cases occurring in January, four in February, three in March and none in April. If one compares the incidence in the vaccinated and unvaccinated members of this group it is seen that 3 or 16.7% of the former were affected as against 12 or 31.0% of the latter. The bare percentages, however, fail to give an accurate idea of the comparative degree of disability in the two groups. All but one of the cases among the unvaccinated were severe. There was marked prostration, high fever (102°-104°F.) and violent local reaction which necessitated hospitalization in every case. The three cases among the vaccinated, on the other hand, were so mild as to be almost subclinical. It seems of importance briefly to describe them.

Case 27.—Med. No. 48784. H. G. white, female, nurse, *æt.* 28. The history was unimportant save for "innumerable" attacks of tonsillitis in the past. On the evening of January 21, she noticed a mild sore throat but worked on the following day in spite of slight chilliness and aching. On the morning of the 23rd she was sent into the ward more for study than because of disability. At that time the throat was sore but she did not feel badly otherwise. On examination the pharynx was diffusely reddened. Both tonsils were ragged and slightly enlarged. "In the center of each is a large crater lining which is a diffusely distributed exudate, grey white in color. No swelling of the follicles." On January 25th the throat was clear. Leucocytes (Jan. 23) 8,500; Jan. 24, 7,700. Highest daily temperature (rectal)—99.8°F. (Jan. 23), 99° (Jan. 24), 99.4° (Jan. 25), 98.6° (Jan. 26). Throat cultures (Jan. 23)—almost pure culture beta-hemolytic streptococcus.

Case 31.—Med. No. 48836. B. G. white, female, nurse, *æt.* 27. Past history unimportant. On January 27th she was taken with malaise, severe general aching, and cough, but remained on duty until January 30th, when she felt too badly to work. On entry to the ward the temperature was 100.5 F.; she complained of intense general aching, there was marked conjunctivitis with photophobia, and frequent dry cough. The throat was not sore. The tonsils were small and ragged but clean, and no glands were palpable. The leucocytes were 4000.

The case was evidently one of influenza, a disease which was epidemic at the time.

A throat culture made on January 31st showed the usual throat flora with a very few colonies of beta hemolytic streptococcus.

On February 1st the patient was practically well but later in the day complained of sore throat. A typical follicular tonsillitis developed with a pure culture of beta hemolytic streptococcus. The attack, however, was

very mild; on February 3rd the throat was clean and on February 6th she was discharged.

Daily leucocyte counts beginning Feb. 1 were as follows: 6,000, 7,800, 12,000, 11,000, 10,000, 9,000. The highest daily temperature (rectal, F.) was 100.5°F. (Jan. 30), 99.5° (Jan. 31), 99.2° (Feb. 1), 100° (Feb. 2), 99° (Feb. 3), and 98.6° (Feb. 4).

CASE 34.—S. white, female, nurse, *æt.* 24. Past history unimportant. On February 23rd the throat felt sore and there was some pain on swallowing. Temperature 99.4 F. Feb. 24th, she felt better, temperature 99.° February 26th, she felt perfectly well; throat no longer sore. The patient was seen first on this day. The tonsils were large and slightly injected. There was no exudate. Culture yielded many colonies of beta hemolytic streptococcus. The patient was at no time off duty.

A consideration, then, of these three cases of tonsillitis in vaccinated nurses indicates the extreme mildness of the attack. S. (Case 34) was not off duty at all; H. G. (Case 27), who reported only at our request, had no constitutional symptoms, no leucocytosis, and practically no fever, and B. G. (Case 31) developed a very mild tonsillitis in connection with an attack of influenza.

On comparing the number of days off duty among the nurses of the vaccinated and unvaccinated groups, one finds a total of about four days among the former as against over 100 days among the latter. If the figures for the vaccinated are multiplied by two to allow for the difference in size of the groups (18,39) it is still evident that over twelve times the number of days was lost by the unvaccinated.

SUMMARY

In another paper [1] detailed evidence is presented which shows that carriage of beta hemolytic streptococcus in the tonsil is associated with a high degree of resistance to acute tonsillitis. It was pointed out further that such resistance depended directly on the presence of the streptococci and that susceptibility was rapidly re-established if the carrier state terminated. The present experiment was carried out because there seemed to be a rational basis for prophylactic vaccination and because in spite of the small number of available subjects accurate bacteriological control was possible. The theoretical consideration of resistance to tonsillitis received further support from the freedom from disease in tonsil carriers of beta hemolytic streptococcus whether vaccinated or unvaccinated. In the case of the non-carriers, who were regarded as susceptible, the protective power of the inoculation is suggested by the lower incidence among the vaccinated and above all by the extremely mild form which the disease took in those who did develop infection.

REFERENCE

1. Bloomfield, A. L., and Felty, A. R.: Arch. Int. Med. (in press, September, 1923).

PATHOLOGICAL CHANGES IN THE KIDNEY IN CONGENITAL SYPHILIS

By ERNESTO DE SOUZA CAMPOS

(From the Department of Pathology and Bacteriology, The Johns Hopkins University)

Notwithstanding the fact that several papers on the subject of the anatomical lesions of the kidney in congenital syphilis have been published, this question does not yet seem to be well settled. Indeed, the best and newest books on Pathology contain very little reference to this matter. Probably this is due to the fact that almost always single or only a few cases have been described. On the other hand, the writers do not always agree on many important points. Hochsinger and Hecker, for instance, hold entirely opposite views as to the possibility that congenital syphilis causes a retardation of development of the kidney. The authors even disagree as to the time in which the appositional growth of the organ normally ceases. The opinions also differ concerning the lesions of the renal parenchyma in congenital syphilis. Furthermore, as regards the interstitial changes, many superficial descriptions have been given which do not resemble the picture presented by more recent investigators (Bloch and others who used proper staining methods). We must also bear in mind that many of the cases of syphilitic lesions of the kidney (in congenital syphilis) were reported before a great number of new methods of diagnosis had become known, which made the identification of syphilis more accurate. As a result, the anatomical picture of congenital syphilis is based almost exclusively on the better known lesions of the lung, liver, pancreas and bones. We think, however, that the kidney is one of the organs which best shows syphilitic lesions because of *the characteristic appearance and uniformity of the histological changes which have been found in all of our cases.* Furthermore, the renal tissue offers greater resistance to maceration than many other organs, thus being of great help in the anatomical diagnosis of syphilis in premature babies, often brought for autopsy in a more or less advanced stage of maceration.

For these reasons, it seems that a study of the kidney in a large number of cases of congenital syphilis would

be of interest. Before describing our observations it affords us great pleasure to acknowledge our indebtedness to Dr. W. G. MacCallum for the suggestion of this interesting subject.

Material and Methods.—All of our material comes from the Department of Pathology of the Johns Hopkins Hospital. We examined the kidneys of ninety-four cases of congenital syphilis, twenty-five of which were so badly macerated that no evidence of any structure could be observed. The other sixty-nine cases we classified as follows:

Premature babies	39
Full-term babies, up to 2 days of extra-uterine life	19
Infants from 2 weeks to 5 months old	11
	69

As may be seen on looking at Tables I, II and III, the silver nitrate impregnation (Levaditi method) was employed in fifty-seven cases, in fifty-two of which spirochætes were found. We will not discuss the diagnosis of these fifty-two cases because the findings of spirochætes and of other signs of syphilis do not permit of any doubt. In five cases (Nos. 15, 59, 61, 62, 65), however, no spirochætes could be demonstrated. In spite of this negative result these five may also be included among the syphilitic cases, as we shall show by giving a short report of each one.

Case 15 is that of a premature infant who developed dactylitis and died on the seventh day. The baby's and mother's Wassermanns were positive. The placenta was syphilitic. At autopsy the lung showed pneumonia alba and bronchopneumonia; the liver, areas of well-defined portal cirrhosis; the pancreas, some scarring; the spleen, a greater amount of fibrous tissue than usual; and the kidney, syphilitic nephritis.

Case 59 is that of a child, 5 months old, who had skin lesions widely distributed over the body. Suggestive rhagades were seen around the corners of the mouth. The skin lesions healed upon treatment with arsphenamin. The Wassermann reaction was positive. The autopsy showed syphilitic nephritis, rickets and pneumonoccic infection.

Case 62 is that of a baby, 3½ months old, who had rhagades around the mouth and anus, interstitial keratitis and iritis, generalized syphilitic skin eruptions, enlargement of the liver and spleen. At autopsy, there were found pneumonia alba, diffuse scarring of the spleen, areas of diffuse scar tissue in the liver, syphilitic nephritis, hæmorrhages in the hypophysis and thymus and generalized acute fibrinous pericarditis. The Wassermann reaction was positive.

Case 65 is that of an infant, 2 months old, who had the entire surface of the skin covered with syphilitic lesions. The Wassermann reaction for both mother and baby was positive. The mother had received four treatments for lues before the birth of the child. The autopsy showed pneumonia alba, slight scarring and extensive blood formation in the liver, scarring and myeloid reaction of the spleen, syphilitic nephritis, extensive myeloid formation (with numerous megalocaryocytes) in the bronchial and mesenteric lymph-nodes, and syphilitic osteochondritis.

In all five cases the kidney showed the lesions which will be described later.

We do not know why spirochætes were not found in these five specimens. It was not on account of maceration because the tissues were well preserved, whereas in macerated babies the spirochætes were found when autopsies were performed from three to six days after death. Noguchi also states that the silver nitrate impregnation for the demonstration of *Treponema pallidum* and *Treponema cuniculi* gives inconstant results. It seems then as though the failure to demonstrate them might have been due to an occasional fault in the technic.

We must now take into consideration twelve cases in which the Levaditi method for the demonstration of spirochætes was not employed. Many of these autopsies were performed before 1907, when this technic (1906) was not known, or, at least, had not been adopted as a routine method. A few of the autopsies were performed before the discovery of *Treponema pallidum* (1905). The diagnosis of congenital syphilis, in these twelve cases, was made on the basis of the clinical history and the anatomical findings. Condensed in Table IV are the most striking features of the anatomical changes in them.

The analysis of all our cases leads us to conclude that all our specimens of kidneys are from cases of congenital syphilis, and this assumption permits us to draw conclusions regarding the lesions which will be described later.

The Wassermann test was made in fifty-four of the sixty-nine cases. The results of this blood test are shown in Table V. All the specimens of kidneys were fixed in Zenker's fluid (Helly's formula). Many were also fixed in ten per cent formaldehyde to be used for the Levaditi silver nitrate impregnation. The Levaditi sections of several cases were also stained with hæmatoxylin-picric-acid-acid fuchsin, or with hæmatoxylin and eosin, in order to demonstrate the location of the spirochætes in the tissues. Routinely, the following staining methods were employed: Hæmatoxylin-eosin, Wright's and Wilson's stains for blood cells and Pappenheim's methyl-green-pyronin. For the oxydase reaction the Goodpasture technic was used.

As control we used forty-three normal kidneys (Tables VI and VII). Syphilis may be entirely excluded from these forty-three cases, because the clinical history and the findings at autopsy show no sign of it. The Wassermann test, when done, was negative (Tables VI and VII). In twenty-three of the forty-three cases we had the oppor-

tunity of examining sections of several organs routinely treated by the Levaditi impregnation method, and no spirochætes were found (Tables VI and VII). These specimens of normal kidneys came from babies who died of birth injury, intra-uterine asphyxia, post-mortem Cæsarian section, bacillary dysentery and so on. Three of the specimens came from the Department of Embryology. We must also mention that in all cases a thorough examination of the kidneys showed no lesions, with the exception of some post-mortem changes.

In order to make clear the report of our results we will first deal with the question of retardation of development of the kidney in congenital syphilis. The histological changes will then be described.

Retardation of Development

It seems that almost all the authors are of the opinion that congenital syphilis causes a retardation of development of the fœtus. Some fœtal conditions frequently found in the liver and pancreas of full-term syphilitic infants have been explained on the basis of this theory. In this manner has been explained the persistence of an extensive blood formation in the liver, often seen a long time after the birth of full-term syphilitic babies. In a similar manner it has also been held responsible for the connections of the islands of Langerhans with the branches of the pancreatic duct. In regard to the retardation of development of the kidney little is known. It will, therefore, be very interesting to see whether there is a true retardation of its development in syphilitic infants. This problem may be approached by the study of the growth of the uriniferous tubules during fœtal and post-fœtal life. It is well known that beneath the capsule of the fœtal kidney there is a continuous layer of metanephrogenic tissue which is usualy called the *neogenic zone*. This metanephrogenic tissue forms, by apposition, several generations of uriniferous tubules. The neogenic tissue regenerates each time from what remains after the formation of the last generation and is pushed toward the periphery of the kidney by the new-growing tubules. As a result of this process, each new layer of uriniferous tubules is covered peripherally by a cap of metanephrogenic tissue, which later forms the next generation, and so forth. This process of appositional growth repeats itself until all the neogenic zone is exhausted and vanishes.

The further growth of the kidney depends, principally, upon the development of the tubules already formed. This being known, it is therefore of great interest to determine when the appositional growth of the normal kidney ceases. This may be ascertained by the disappearance of the neogenic zone. Some misunderstanding still exists as to the exact time in which the neogenic zone is no longer seen in the cortical region of the kidney. Felix (Keibel and Mall) states that "the formation of the uriniferous tubules is not completed at the close of the fœtal period, but extends into the first days of the extra-uterine life." He thinks that the formation of new tubules ceases after the tenth day of extra-uterine life. Aschoff (1911) says that the neogenic zone is still present in the subcapsular layer of the kidney up to the third or sixth month after birth. Keith points out that, up to the time of birth, tubular and glomerular formations are seen in full activity within the subcapsular zone of the kidney; soon after birth, the formation of new elements ceases. Hecker is of the opinion that the peripheral metanephrogenic tissue decreases with the growth of the infant, not according to rule but depending upon the energy of the growth. He thinks that the kidney, at the time of birth, has not finished its appositional growth, so that the presence of the neogenic zone, before or after birth, is entirely normal. Toldt came to the conclusion that in man and in dogs the glomeruli are still being formed after eight or ten days of extra-uterine life. Prentiss and Arey hold the same opinion. Huber has observed in rabbits the new formation of tubules as late as the first week after birth. In cats, he found newly-forming renal vesicles a short time before birth. The same author thinks the period in which the formation of renal vesicles ceases varies somewhat in different animals. On the other hand, Herring, as Huber quotes, states that no more tubules are formed in the kidney after the eighth month of fœtal life. Stoerk, who studied the question broadly, is of the same opinion. Hochsinger, also, believes that at the time of birth all the uriniferous tubules are already formed.

On account of the various views of the authors on this subject, new observations upon the conditions of the neogenic zone in fœtal and post-fœtal life, are needed to determine the time at which the new formation of the uriniferous tubules ceases. It is also important to determine this in human beings, because it seems to vary in different animals. With this in mind, we examined normal kidneys from forty-three infants, of which twenty were premature and twenty-three were born at term. Eleven of the full-term babies were stillborn and the others lived from seven hours to six months (Tables VI, VII). The results of the investigation show a sharp distinction between the fœtal and post-fœtal kidneys. All the kidneys of premature babies, from the fifth up to the eighth or ninth month of fœtal life, show the subcapsular zone of metanephrogenic tissue (Table VI). On the other hand, in all the kidneys of full-term infants, there is no evidence of a neogenic zone (Table VII). Thus, we must come to the conclusion that the uriniferous tubules are practically all formed at the time of birth and that the further development of the kidney depends upon the growth of the tubules already formed. It may then be inferred that the presence of the neogenic zone in the kidneys of full-term babies indicates a retardation of development of the organs. Table I shows the results of

the study of the kidneys of thirty-nine syphilitic premature babies. The neogenic zone is present in every case. Table II presents the results of the examination of kidneys of syphilitic babies born at term. The subcapsular metanephrogenic zone is still present in all cases, with the exception of cases No. 55 and No. 57. Table III shows that the neogenic zone is not present in the kidney of all eleven infants from the age of two weeks to five months. Unfortunately, in the sixty-nine cases, there are no full-term babies between the age of two days and two weeks, thus preventing us from observing how long the neogenic zone persists in kidneys of syphilitic infants.

In summing up these results we may conclude that in almost all the cases of congenital syphilis the kidney is retarded in its development. This conclusion agrees with the views of Hochsinger which have been criticized by Hecker.

Histological Lesions

We shall now describe the interstitial changes of the kidney in congenital syphilis and afterwards the lesions of the renal parenchyma.

Interstitial Changes.—From the very first observations a striking histological picture was seen. The same condition was later found in all sixty-nine cases in greater or lesser degree. This histological change is mainly represented by an accumulation of cells around the blood vessels of the cortical region of the kidney. In very slight cases this infiltration is almost entirely limited to the adventitia of the blood vessels, principally that of the larger vessels ("arcuate vessels") between the cortical and medullary zone. In Cases 21, 49 and 55, for instance, only the "arcuate" vessels show a perivascular infiltration, and in Cases 15, 51, 67 this infiltration is more conspicuous around these vessels. There are cases, however, in which the cellular new growth is more pronounced around the smallest cortical arterioles (Nos. 7 and 23) and sometimes the latter are the only vessels involved (Nos. 4 and 52). In more advanced cases the cellular proliferation goes further, pushing apart tubules and glomeruli, and forming a thick layer around the blood vessels. Furthermore, the cellular growth infiltrates the interstitial tissue between neighboring tubules and glomeruli, which finally are embedded in this large cellular mass. In extreme cases every blood vessel of the cortex is involved: the cells are arranged in a continuous sheet along the interlobar, "arcuate," and interlobular vessels, following the smallest cortical arterioles, and even the *vasa afferentia* of the glomeruli. Figure I is a microphotograph of the kidney from Case 40, in which a branching cortical arteriole is seen enveloped by a conspicuous layer of cells. Figure II, from the same case, shows a tremendous infiltration around an arcuate vessel; a glomerulus is seen embedded in this cellular tissue.

In the neogenic zone, occasionally, there are scattered cells, which present the same structure as those seen about the blood vessels.

In sharp contrast to the extensive cortical lesions, the medulla of the kidney shows very slight changes. In only seven out of sixty-nine cases, small collections of

TABLE I.

Premature Infants.

No.	Autopsy No.	Age	Mother's Wa.R.	Baby's Wa.R.	Levaditi	Neogenic zone	Perivascular infiltration of cortex	Medulla	Pelvis
1	5884	5 mos.	+	O	+	+	Conspicuous	Inc. str.	N.C.
2	7091	7 mos.	—1	O	+	+	Pronounced	N.C.	Infilt.
3	6965	7½ mos.	—	—	+	+	Slight	N.C.	Infilt.
4	6955	7 mos.	+	O	+	+	Pronounced	N.C.	N.C.
5	6375	7 mos.	—	O	—	—	Conspicuous	N.C.	O
6	6324	7 mos.	+	O	+	+	Conspicuous	N.C.	O
7	6128	7 mos.	—	+	+	+	Pronounced	N.C.	Infilt.
8	5870	7 mos.	O	+	+	+	Pronounced	N.C.	Infilt.
9	5588	7 mos.	+	+	+	+	Conspicuous	N.C.	N.C.
10	5214	7 mos.	+	O	+	+	Pronounced	N.C.	O
11	4483	7-8 mos.	+	O	+	+	Pronounced	N.C.	N.C.
12*	6543	8 mos.	+	+	+	+	Pronounced	Casts	Infilt.
13	6484	8 mos.	?	O	+	+	Pronounced	Inc. str.	O
14	6212	8 mos.	+	O	+	+	Conspicuous	N.C.	Infilt.
15	6087	8 mos.	+	+	—	+	Conspicuous	N.C.	N.C.
16	5978	8 mos.	?	—	+	+	Conspicuous	N.C.	O
17	5561	8 mos.	+	O	+	+	Pronounced	N.C.	N.C.
18	5560	8 mos.	—	O	+	+	Pronounced	N.C.	O
19	5485	8 mos.	+	O	+	+	Slight	N.C.	N.C.
20	4632	8 mos.	+	O	+	+	Pronounced	N.C.	Infilt.
21	4468	8 mos.	O	O	O	+	Pronounced	N.C.	O
22	2297	8 mos.	O	O	O	+	Pronounced	N.C.	Infilt.
23	6970		+	+	+	+	Pronounced	N.C.	N.C.
24	6878		—	O	+	+	Pronounced	N.C.	O
25	5760		—	O	+	+	Slight	N.C.	O
26	5738		O	O	+	+	Pronounced	N.C.	O
27	5553	9 mos.	+	O	+	+	Conspicuous	N.C.	N.C.
28	5451		+	O	+	+	Pronounced	N.C.	N.C.
29	5446		—	+	+	+	Pronounced	N.C.	N.C.
30	5275	9 mos.	+	O	+	+	Pronounced	N.C.	O
31	5178		—	O	+	+	Slight	N.C.	N.C.
32	5164		+	O	+	+	Pronounced	N.C.	N.C.
33*	4965		—	+	+	+	Conspicuous	Casts	O
34	4559		+	O	+	+	Pronounced	Inc. str.	Infilt.
35	4475		+	O	+	+	Pronounced	N.C.	Infilt.
36	2591		O	O	O	+	Pronounced	N.C.	N.C.
37	5379	9 mos.	O	+	+	+	Conspicuous	N.C.	O
38	5036	9 mos.	?	+	+	+	Slight	N.C.	O
39	4494		+	O	+	+	Conspicuous	Inc. str.	O

* Casts in tubules.
—1 Received antiluetic treatment.
Inc. Str.—Increased stroma.
+—Positive.
"—"—Negative.
O—Not done or not known.
O—Not present in section.
Infilt.—Infiltration of cells.

cells were found scattered here and there, mainly near the cortical zone. The pelvis, however, is frequently affected. An infiltration of the connective tissue of the pelvis is seen in twenty-three out of thirty-seven cases in which this tissue was present in the sections. (Tables I, II and III). In two cases there is also seen a diffuse hæmorrhage in the connective tissue of the pelvis. The capsule of the kidney shows a slight infiltration in several cases (Tables I, II and III) and in one case a diffuse hæmorrhage.

TABLE II.

Syphilitic Babies Born at Term.

No.	Autopsy No.	Age	Mother's Wa.R.	Baby's Wa.R.	Levaditi	Kidney			
						Neogenic zone	Perivascular infiltration of cortex	Medullary zone	Pelvis
40*	7106	5 hrs.	?	+	+	+	Pronounced	Infilt.	Infilt.
41	6360	5 mos.	+	O	+	+	Pronounced	N.C.	Infilt.
42*	5992	S. B.	+	O	+	+	Pronounced	N.C.	Infilt.
43	5811	S. B.	—	O	+	+	Slight	N.C.	O
44	5455	S. B.	+	O	+	+	Conspicuous	N.C.	O
45*	4930	½ hr.	+	—	+	+	Pronounced	6 casts	O
46	4802	S. B.	+	+	+	+	Pronounced	Infilt.	O
47	4602	S. B.	O	O	+	+	Conspicuous	N.C.	O
48*	4493	S. B.	+	+	+	+	Conspicuous	N.C.	Infilt. Hem.
49	4467	S. B.	—	O	O	+	Pronounced	Infilt.	Hem.
50	4461	S. B.	+	+	+	+	Pronounced	N.C.	O
51*	1580	S. B.	O	O	O	+	Pronounced	N.C.	Infilt.
52	2875	30 min.	O	O	O	+	Conspicuous	N.C.	O
53	1381	3 hrs.	O	O	O	+	Pronounced	N.C.	Infilt.
54	1153	3½ hrs.	O	O	O	+	Pronounced	N.C.	O
55	1360	4 hrs.	O	O	O	—	Slight	N.C.	O
56	2852	1 day	O	O	O	+	Pronounced	N.C.	Infilt.
57	2976	1 day	O	O	O	—	Pronounced	N.C.	O
58	6740	2 days	O	O	+	+	Pronounced	N.C.	O

*—Slight parenchymatous changes.
N. C.—No changes or postmortem changes.
+—Positive.
"—"—Negative.
?—Doubtful.
O—Not done or not known.
O—Not present in section.
S. B.—Stillborn.
Infilt.—Infiltration of cells.
Hem.—Hæmorrhage.

A review of the literature indicates that a cellular infiltration in kidneys of syphilitic infants has been described by several investigators.

In 1875, Coupland reported to the Pathological Society of London, the results of the microscopical observation of the specimens from a case of congenital syphilis. In the kidney he found "a massing of nuclei around vessels and between the tubules, in the cortical portion." Maffucci, as Marchiafava quotes, made the same statement in a case of a syphilitic fœtus.

In 1884, Marchiafava described the kidney of a syphilitic, full-term, stillborn infant, in which he found an extensive perivascular infiltration throughout the cortex. There were no changes in the medulla.

Stroebe, in 1891, noticed the same thing in a syphilitic baby born three weeks before full development.

In 1898 and 1900, Hecker published two papers on the pathology of congenital syphilis. He described in the kidney an interstitial small-cell infiltration, mainly about the blood vessels.

TABLE III.

Syphilitic Infants from Two Weeks to Five Months Old.

No.	Autopsy No.	Age	Mother's Wa.R.	Baby's Wa.R.	Levaditi	Kidney				
						Neogenic zone	Perivascular infiltration of cortex	Medulla	Pelvis	Parenchymatous changes
59	6914	5 mos.	O	+	—	—	Conspicuous		Infilt.	+
60	6587	4 mos.	O	+	+	—	Pronounced	Infilt.	O	+
61	6228	3 mos.	O	+	—	—	Pronounced		Infilt.	+
62	6563	3½ mos.	O	+	—	—	Conspicuous		Infilt.	+
63	5335	13 wks.	+	+	+	+	Slight	Casts	O	+
64	6417	2½ mos.	O	O	+	—	Pronounced	Infilt.	Infilt.	+
65	7114	2 mos.	+	+	—	—	Pronounced	Infilt.	Infilt.	+
66	4629	2 mos.	O	O	+	—	Conspicuous	Infilt.	O	+
67	6951	7 wks.	+	+	+	—	Pronounced		Infilt.	+
68	5210	1 mo.	O	+	+	—	Pronounced		O	+
69	4414	2 wks.	+	O	O	—	Pronounced		O	—

+—Positive.
"—"—Negative or not present.
O—Not done or not known.
O—Not present in section.
Infilt.—Infiltration of cells.

In the same year, Haushalter and Richon observed in the kidney of a nine months old syphilitic baby the cortical arterioles surrounded by a sheet of cells.

Hochsinger, in 1898, gave a minute description of the pathological changes observed in the organs of five syphilitic babies. He described an interstitial cellular infiltration in the cortex of the kidney; the medullary zone showed very slight changes. An interstitial cellular infiltration of the cortex of the kidney has been also described by Cassel (1904) and Hahn (1912) in several cases.

In 1920 Frazer found the same condition in a syphilitic infant.

The infiltration above described shows a great variety of cells. In order to make a cellular study, twenty-four well preserved specimens were selected (Nos. 4, 8, 12, 16, 21, 23, 24, 26, 33, 36, 40, 41, 49, 52, 53, 56, 57, 58, 60, 61, 64, 65, 67 and 69). Sections from these specimens, measuring from 5 to 7 micra, were stained with Pappenheim's methyl-green-pyronin and with Wright's and Wilson's stain for blood cells. With Pappenheim's stain

the new-formed cells fail to show the red color of the cytoplasm which occurs in the plasma cells and lymphocytes. On the other hand, with Wright's and Wilson's stain these cells show the cytological structure of the bone-marrow cells, i. e., *non-granulated* (No. III, Fig. III) *myeloid cells* (myeloblasts of Schridde and Naegeli, leucoblasts of Pappenheim, hæmocytoblasts of Ferrata); neutrophilic (No. II, Fig. III) and eosinophilic (No. I, Fig. III) *myelocytes; normoblasts* (No. IV, Fig. III) with basophilic and acidophilic cytoplasm; *megalocaryocytes* (Fig. III, above to the left). The megalocaryocytes were seen in eight specimens (Nos. 4, 12, 16, 40, 60, 61, 64, 65). These megalocaryocytes have various nuclear structures such as Firket and Campos have described in the tissues of rabbits, after saponin poisoning, i. e., large nuclei, very complicated in structure, which have been compared to a "basket-work," composed of threads twisted in the most complex way; large and small nuclei so rich in chromatin that the entire nuclear body is a dense mass very deeply stained by the basic dyes (Fig. III, above to the left). Some of these megalocaryocytes have a cytoplasm apparently homogeneous and of a light basophilic color. Others show a delicate granulation of a lilac or violet color. Many of the immature granulocytes show mitotic figures. No basophilic myelocytes were observed among the cells of the perivascular infiltration. Besides the early myeloid elements a few mature blood cells may be seen.

Figure III shows a drawing of a microscopical field of the cortex of the kidney (No. 64). A transverse section of a cortical arteriole (interlobular) is shown surrounded by a tremendous myeloid infiltration.

The oxydase reaction done in two cases gave positive results.

A few investigators, who used proper staining methods, found in a few cases of congenital syphilis a myeloid formation in the kidney. The same picture has also been found by a few German investigators (Schridde, Fischer, Rautmann) in cases they called congenital general dropsy (angeborener fötaler allgemeiner Wassersucht).

Swart (1905) reported four cases which he labeled "Bantische Krankheit? Syphilis?" In a case of a newborn child he described cirrhosis and blood formation in the liver and a perivascular myeloid infiltration in the cortex of the kidney.

Schridde, in 1906, reported two cases, one of which (the second case), he said, was undoubtedly one of congenital syphilis. In the first case he found cirrhosis and blood formation in the liver, extensive myeloid proliferation in the cortex of the kidney and myeloid metaplasia of the lymph-nodes. In the second case he described an extensive myeloid formation in the liver, kidney and connective tissue of the vaginal wall.

In 1910, 1911, Schridde described several cases under the title of "Congenital General Dropsy," in which he found blood formation in the spleen, liver and kidney. He was uncertain about the etiology but excluded syphilis, as he did not find spirochætes, and the clinical history was negative.

In 1912 Fischer reported two cases, under the same title. In the first case he found scarring and blood formation in the liver and a perivascular blood formation in the cortex of the kidney. In the second case the Wassermann was positive. There was interlobular scarring and extensive blood formation in the liver and around the cortical vessels of the kidney.

In the same year, Rautmann presented another case of "Fötaler allgemeiner Wassersucht" in which he saw blood formation in the liver, spleen and kidney (perivascular). In 1920, Black minutely described a case of a six weeks old child with congenital syphilis. He described blood formation in the liver, spleen and kidney (around the blood vessels) and also syphilitic osteochondritis. It seems as though all these were instances of congenital syphilis, because the negative result (in several cases) of the search for spirochætes is not a good reason for excluding the possibility of syphilis, when other findings such as scarring and extensive blood formation seem to indicate it.

The myeloid formation, above described, seems to be caused by the presence of spirochætes in the renal tissue. Indeed, spirochætes are seen almost exclusively in the interstitial tissue of the kidney, mainly about the blood vessels and among the infiltrating cells. This fact is evident when the silver-nitrate-impregnated tissue is counterstained with hæmatoxylin, picric acid and acid fuchsin or with hæmatoxylin-eosin. The spirochætes are also seen in greater numbers in the cortex than in the medullary zone of the kidney. At times in the medullary zone they are only rarely seen and their number always decreases towards the papilla. In extreme cases the spirochætes are grouped in such tremendous numbers about the blood vessels that with the low power they appear as a black layer (Levaditi method) following the branching of the cortical vessels. The spirochætes may also be seen in the tubules, glomeruli and lumina of the blood vessels but in smaller numbers. It is also interesting to note that Warthin found that "the spirochætes are present in the kidney in greater number, even more so than in the liver."

The myeloid formation in the kidney, in congenital syphilis, cannot be the result of the retardation of development of the organ. It has been shown that the kidney normally is not a blood-forming organ as are the liver, spleen, bone marrow and lymph-nodes. Danchakoff points out that neither in birds nor in mammals does the stroma of the kidney show a hæmopoietic activity, even in embryonic life. Bloch also states that, according to recent investigations, the kidney is never a fœtal blood-forming organ, except in the earliest stages of embryonic life, when a hæmopoiesis may be considered as taking

TABLE IV.

Lesions in the organs in the cases of congenital syphilis in which no Levaditi sections were made.

Case No.	Autopsy No.	Lung	Liver	Pancreas	Kidney	Bone
21	4468	Increased interalveolar tissue	Scarring and blood formation	Extensive scarring	Perivascular infiltration of cortex	No section
22	2297	Scarring	Scarring and blood formation	Scarring	Perivascular infiltra- of cortex	No section
36	2591		Scarring and blood formation	Extensive scarring	Perivascular infiltra- of cortex	No section
49	4467	Increased interalveolar septa. Perivasc. infilt. of immature cells	Blood formation	Scarring	Perivascular infiltra- of cortex	No section
51	1580	Increased interalveolar septa.	Scarring	No section	Perivascular infiltra- of cortex	No section
52	2875	Increased interalveolar septa.	Blood formation	Extensive scarring	Perivascular infiltration of some cortical arterioles	No section
53	1381	Pneumonia alba	Scarring and blood formation	No section	Tremendous perivascular infilt. of cortex	Syphilitic osteochondritis
54	1153	Interstitial pneumonia	Scarring and blood formation	No section	Tremendous perivascular infilt. of cortex	Syphilitic osteochondritis
55	1360	Pneumonia alba	Scarring and extensive blood formation	Extensive scarring	Slight perivascular infiltration	No section
56	2852	Pneumonia alba	Blood formation	Scarring	Tremendous perivascular infiltration	No section
57	2976	Pneumonia alba	Blood formation	No section	Perivascular infiltration of cortex	Syphilitic osteochondritis
69	4414	Increased interalveolar septa	Scarring Blood formation	No section	Pronounced perivasc. infiltration of cortex	Syphilitic osteochondritis

place everywhere in the blood sinuses and capillaries. In our series of twenty normal kidneys of premature babies from the fifth to the ninth month of fœtal life, we did not see a picture of hæmopoietic activity. The hypothesis that all the extra-medullary blood formation is due to metastasis from bone-marrow cells finds no basis in the history of the origin of the blood and blood-forming organs, because the myeloid cells are present in the spleen and liver before the bone-marrow is formed. It seems that all the probabilities are in favor of an extra-vascular blood formation in the kidney in congenital syphilis, as the numerous mitotic figures in the myeloid cells seem to indicate. It has been recently proven (Danchakoff, Sabin) that the granulocytes, the forerunners of the leucocytes, have always an extra-vascular origin. Ferrata states that the connective tissue has a hæmopoietic activity, normally very slight, but which can be accentuated in pathological conditions or under experimental stimuli. Sabin points out that in the embryo the origin of blood from connective tissue is very widespread and can be made much more so by certain experimental stimuli. Danchakoff made the statement that "descriptive investigations in embryology and pathology have furnished numerous data concerning a possible polyvalency" [regarding the blood formation] "of the loose mesenchyma and of the stroma of various organs in embryonic and adult life." The same author demonstrated experimentally an extensive granulopoietic activity almost everywhere in the mesenchyma of the embryo, after grafts of adult tissue on its allantois. The myeloid formation was found in the stroma of muscles, tendons, sex-glands, liver, pancreas, kidneys, adrenals, and so on.

As it seems that under certain stimuli the connective tissue, and especially the perivascular adventitia, may form blood cells, it is probable that the presence of spirochætes in the interstitial tissue of the kidney furnishes the stimuli for this hæmopoietic activity.

PARENCHYMATOUS LESIONS

Notwithstanding the extensive interstitial changes, the renal parenchyma is almost always well preserved in premature or full-term stillborn babies. In thirty-nine premature babies only two showed hyaline casts in several convoluted and collecting tubules. Hyaline casts were also seen in three of the full-term stillborn babies. In one case (No. 48) there were two small cysts, about 3 or 4 mm. in diameter, which were lined with flat epithelium.

On the other hand, in all children who lived up to five months, a greater or lesser degree of parenchymatous alteration has been observed. In all these cases several tubules are filled with hyaline material. The glomeruli are affected in five cases. There may be seen thrombi of the capillaries, adherence to the Bowman's capsule, or the entire invasion of the glomeruli by dense fibrous tis-

TABLE V.

Cases with positive information as to relation of spirochætes to Wassermann reaction.

	Total cases	Baby's Wa. R.		Spirochætes	
		Positive	Negative	Positive	Negative
Mother's Wa. R. Positive...	11	10	1	9	2
Mother's Wa. R. Doubtful...	3	2	1	3	
Mother's Wa. R. Negative...	4	3	1	4	
Baby's Wa. R. Positive.....	22			17	5
Baby's Wa. R. Negative.....	3			3	

Many other cases are incomplete.

sue. Almost always the "serous space" of the Bowman's capsule contains albuminous material.

The oldest children present gradually more extensive parenchymatous change. These observations confirm those of Hecker, who states that the degenerative processes in the epithelium come to the foreground only in full-term babies, but that altogether these changes are not developed to a very high degree. He also points out that greater parenchymatous changes are to be observed only in infants who have had an extra-uterine life. The same author, however, states that the interstitial changes gradually disappear with the growth of the infants and with the increase in intensity of the degenerative phenomena. We cannot agree with this opinion, because in all our eleven cases of children from two weeks to six months old the infiltration is pronounced or, at least, very conspicuous. Case 63 alone shows a slight infiltration

TABLE VI.

Normal premature babies.
Neogenic zone of the kidney.

No.	Age	Mother's Wassermann Reaction	Levaditi Sections	Neogenic Zone
1	5 mos.	Negative	No spirochætes found	Present
2	5 mos.	Negative	No spirochætes found	Present
3	5 mos.	Negative	No spirochætes found	Present
4	5½ mos.		No spirochætes found	Present
5	5½ mos.	Negative	No spirochætes found	Present
6	6 mos.	Negative	No spirochætes found	Present
7	6 mos.		No spirochætes found	Present
8	6 mos.	Negative	No spirochætes found	Present
9	6 mos.	Negative	Not done	Present
10	6½ mos.	Negative	No spirochætes found	Present
11	6½ mos.	From the Embryological Dept.		Present
12	7 mos.	Negative	No spirochætes found	Present
13	7 mos.	Not done	Not done	Present
14	7 mos.	From the Embryological Dept.		Present
15	8 mos.	Not done	No spirochætes found	Present
16	8 mos.	Negative	No spirochætes found	Present
17	8 mos.	Negative	Not done	Present
18	8 mos.	From the Embryological Dept.		Present
19	8-9 mos.	Negative	No spirochætes found	Present
20	8-9 mos.	Negative	No spirochætes found	Present

around a few blood vessels, but this has also been observed in premature babies. Hecker himself described the kidney of a five and a half months old girl in which he found an extensive perivascular infiltration, besides degenerative processes.

Maceration

As we have already mentioned, the renal tissue offers great resistance to maceration. In twelve of our cases the lung, liver and pancreas were entirely macerated but the kidney, although somewhat autolysed, remained still in quite good condition. This confirms, in so far as the kidney is concerned, the recent study of Lewis and McCoy on the survival of cells after death of the organism. In

TABLE VII.

Normal babies born at full-term.
Neogenic zone of the kidney.

No.	Age	Mother's Wassermann Reaction	Levaditi Sections	Neogenic Zone
1	Stillborn	Negative	No spirochætes found	Not present
2	Stillborn	Negative	No spirochætes found	Not present
3	Stillborn	Negative	Not done	Not present
4	Stillborn	Not done	No spirochætes found	Not present
5	Stillborn	Not done	No spirochætes found	Not present
6	Stillborn	Not done	No spirochætes found	Not present
7	Stillborn	Negative	Not done	Not present
8	Stillborn	Negative	No spirochætes found	Not present
9	Stillborn	Negative	Not done	Not present
10	Stillborn	Not done	Not done	Not present
11	Stillborn	Not done	Not done	Not present
12	7 hrs.	Not done	Not done	Not present
13	8 hrs.	Negative	No spirochætes found	Not present
14	41 hrs.	Not done	Not done	Not present
15	1½ days	Not done	Not done	Not present
16	2 days	Negative	Not done	Not present
17	3 days	Negative	No spirochætes found	Not present
18	3 days	Negative	Not done	Not present
19	4 days	Not done	Not done	Not present
20	14 days		No spirochætes found	Not present
21	1 month	Not done	Not done	Not present
22	5 weeks	Negative	Not done	Not present
23	6 months	Not done	Not done	Not present

a list of twenty-seven different kinds of cells those of the kidney occupy the third place, the first being the macrophages, which they found to be the most resistant. The lung epithelium occupies the fourteenth place, the pancreas cells the eighteenth and the liver cells the twentieth. As far as we could learn in our microscopical sections, the cells of the convoluted tubules are less resistant than those of the straight tubules and glomeruli. Warren and Whipple, also, in an experimental study of the resistance of several tissues to autolysis, found the cells of the convoluted tubules to be the most sensitive of the renal parenchyma; the straight, collecting tubules and

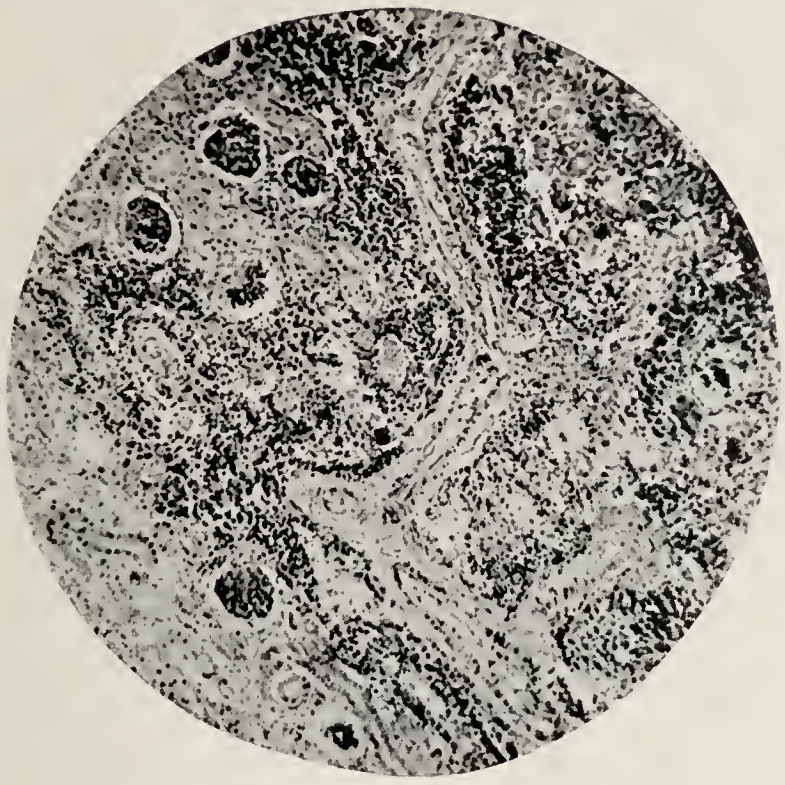

Fig. 1.

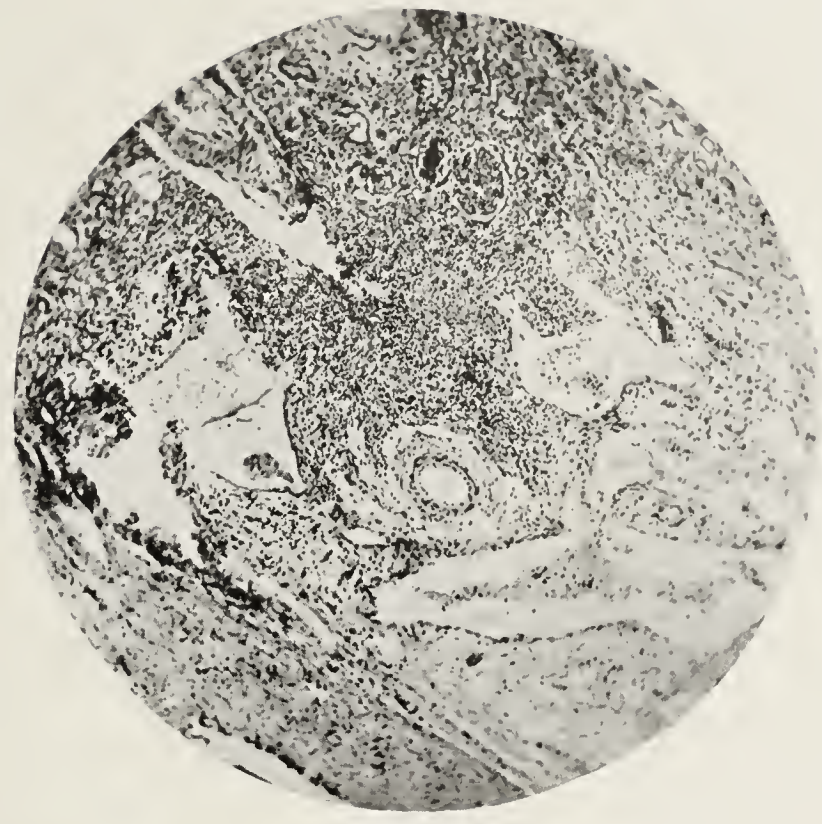

Fig. 2.

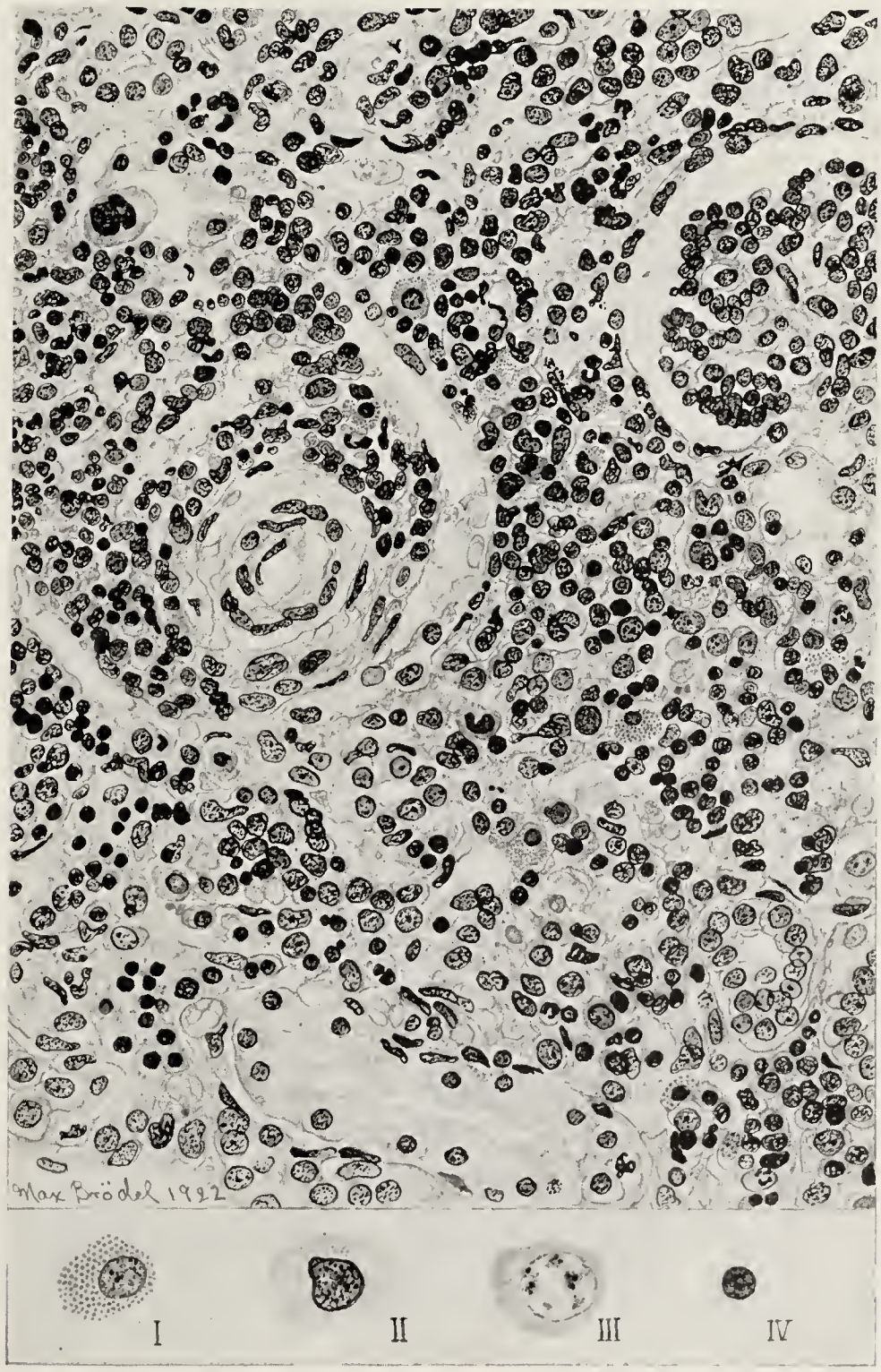

Fig. 3.

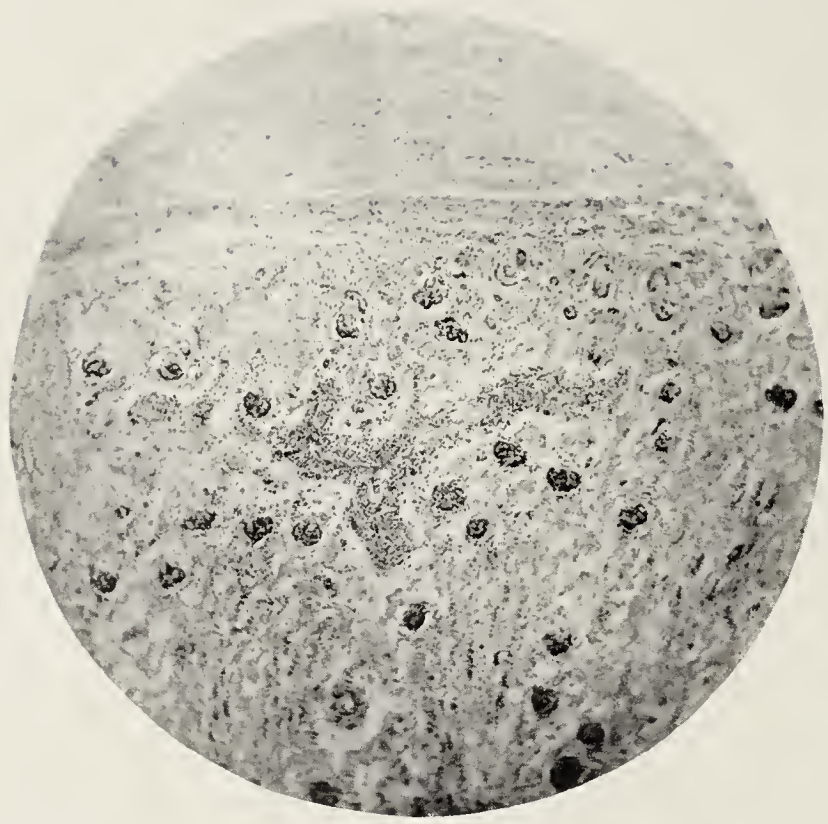

Fig. 4.

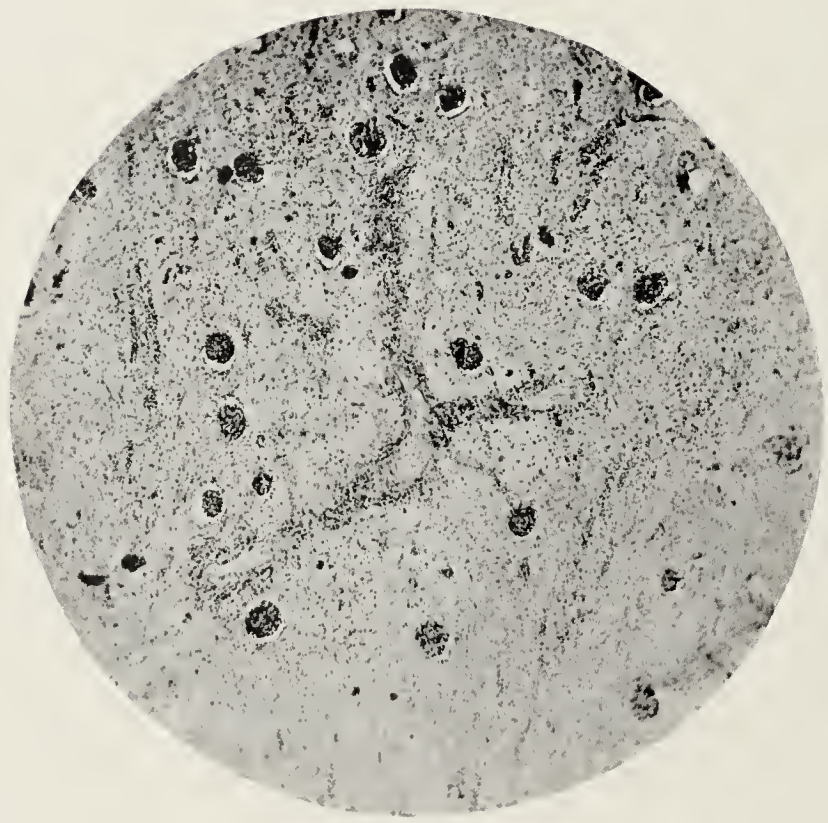

Fig. 5.

glomeruli are more slowly digested. The cells of the perivascular infiltration possess greater resistance than all the other cells of the renal parenchyma. This fact is of great importance for the histological diagnosis of Congenital Syphilis.

Figs. IV and V show microphotographs of a macerated kidney in which the perivascular infiltration is clearly seen.

Conclusions

1. A microscopical examination of the kidneys from sixty-nine cases of congenital syphilis showed histological changes in this organ in every case.

2. These histological changes were observed in the interstitial tissue and, to a lesser degree, in the parenchymatous apparatus.

3. The interstitial changes which were observed in every case consist principally of a cellular infiltration around the blood vessels of the cortex of the kidney.

4. These changes were very pronounced in forty-three cases, conspicuous in eighteen and slight in eight.

5. The medullary zone of the kidney is rarely affected and then in very slight degree. Only in seven out of sixty-nine cases were a few collections of cells observed and then principally near the cortex.

6. The pelvis of the kidney is frequently infiltrated with cells which have the same structure as those seen around the blood vessels.

7. The capsule of the kidney shows only a slight infiltration in a few cases.

8. The cells of the infiltration of the kidney in congenital syphilis are similar in structure to the bone-marrow cells, namely, they are *non-granulated* myeloid cells (myeloblasts of Schridde and Naegeli, leucoblasts of Pappenheim, hæmocytoblasts of Ferrata), *neutrophilic* and *eosinophilic myelocytes, normoblasts* and *megalocaryocytes* (the last observed in eight specimens).

9. These perivascular myeloid cells found in the kidney, in congenital syphilis, seem to have an autochthonous origin, as they show numerous mitotic figures; this fact may be explained on the basis of the extravascular origin of the granulocytes.

10. The formation of the myeloid tissue in the kidney, in congenital syphilis, seems to be stimulated by the presence of spirochætes, because the latter are seen lying in greater numbers in the interstitial tissue of the organ, mainly around the blood vessels.

11. The perivascular myeloid cells offer great resistance to autolysis, thus permitting the microscopical picture of congenital syphilis of the kidney to be seen even in rather advanced maceration. This fact is important for the anatomical diagnosis of congenital syphilis, because premature babies are often brought for autopsy in a more or less advanced stage of maceration.

12. Parenchymatous lesions in the kidney in congenital syphilis, are practically observed only in infants who have had an extra-uterine life and becomes more pronounced as the infants become older. Perhaps it is due to the functional activity of the organ.

13. The kidney in congenital syphilis is retarded in its development. This fact is demonstrated by the presence of the "neogenic zone" in kidneys of full-term, stillborn, syphilitic babies, while this is not to be observed in kidneys of normal, full-term, stillborn infants.

EXPLANATION OF THE FIGURES

Fig. I.—Microphotograph of a blood vessel of the cortex of the kidney (Case 40, H Autopsy 7106). A tremendous infiltration is seen surrounding the branching blood vessel. To the left there is a transverse section of a smaller blood vessel also surrounded by the infiltrating cells.

Fig. II.—Microphotograph of a transverse section of an "arcuate" vessel, from the same case. The blood vessel is surrounded by a large number of myeloid cells. A glomerulus is seen embedded in this cellular mass.

Fig. III.—Drawing of a microscopical field of the cortex of the kidney from Case 64 (Autopsy 6417). Bausch & Lomb, Ocular No. 101, Objective 4 mm. There is seen a transverse section of a cortical arteriole surrounded by an extensive myeloid formation. Above, towards the left, there is a megalocaryocyte. Below, several cells of the myeloid proliferation are represented as seen in an oil-immersion magnification (Ocular No. 10; Objective 1-9mm.)

No. I.—Eosinophilic myelocyte.
No. II.—Neutrophilic myelocyte.
No. III.—Non-granulated myeloid cell (myeloblast).
No. IV.—Normoblast.

Fig. IV.—Microphotograph of the specimen from Case 2 (Autopsy 7091). The kidney is macerated. A branching cortical arteriole is seen enveloped in a cellular infiltration.

Fig. V.—Microphotograph of the specimen from Case No. 11 (Autopsy 4483). The kidney is seen in a more advanced stage of maceration. The perivascular infiltration is still well seen.

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STUDIES ON VIRULENCE

I. AN AUTOMATIC TRANSFERRING DEVICE: INFLUENCE ON VIRULENCE OF GROWTH OF MICROORGANISMS DURING THE LOGARITHMIC INCREASE PHASE

By Lloyd D. Felton

(From the Pathological Laboratory, Johns Hopkins Medical School)

The problem of the disease-producing faculty of microorganisms is one that has claimed the attention of many investigators. Much has been learned concerning the so-called battle between the host and the invading microorganisms,—the susceptibility and resistance of the host, on the one hand, and the virulence of the parasite on the other. The dynamic relationship of these opposed forces has been shown to be variables in producing a constant result,—disease or death of one. It is generally believed and proven that should the host survive a microbic infection, it has undergone some functional change, which, with most microorganisms, is demonstrable by an increased resistance for the specific infecting agent: should the host die, the microorganisms may die with it or perchance be passed on to another animal. In the latter case, the microorganisms, depending upon their state of pathogenicity, whether of low or of high virulence, may have developed an enhanced pathogenic character, or may have remained unaltered, or perhaps even have decreased in ability to infect an animal. The antibiotic relationship becomes more incompatible for host and bacteria in degree depending upon the resistance of the animal and virulence of the microorganism.

Under the conditions of laboratory experiment, in a general way a direct ratio exists between the virulence of the invading organism and the resistance of the host; that is, the more resistant the animal, the more virulent must the bacteria be to cause its death; the weaker this state of resistance, the less virulent the microorganisms may be to accomplish the same result. This relationship

has been extended by experimental methods to include a very wide range of these variables. The defense mechanism of the animal can be so strengthened that relatively enormous doses of the highest virulence may not kill the host. On the other hand, the virulence of a bacterium may be so enhanced that the normal animal is incapable of resisting an infection with a single microorganism. The fluctuating character of the state of virulence of a microorganism either cultivated *in vitro* or passed from animal to animal is an established fact. However, the underlying conditions which cause the alteration of this attribute of bacteria are not definitely understood. Since the memorable observations of Pasteur on the attenuation of the anthrax bacillus, the method he described has been extended to most microbic species. Attenuation further has been accomplished by a variety of methods—high temperature, age, poor media, dryness, variation in interval of transfer, chemical agents, growth in immune sera, etc. No great difficulty has been encountered in decreasing the virulence of microorganisms *in vitro*; in fact, the experience common to all bacteriologists is that an organism, isolated from an animal which has succumbed to an infection, more or less gradually loses its virulence under the conditions of ordinary laboratory methods. Although many hypotheses have been advanced to explain the mechanism of this method of attenuation of virulence, such as lack of fresh animal tissue, acid formation during growth, toxic metabolic products, etc., the real reason for the alteration in this biological activity is not clear.

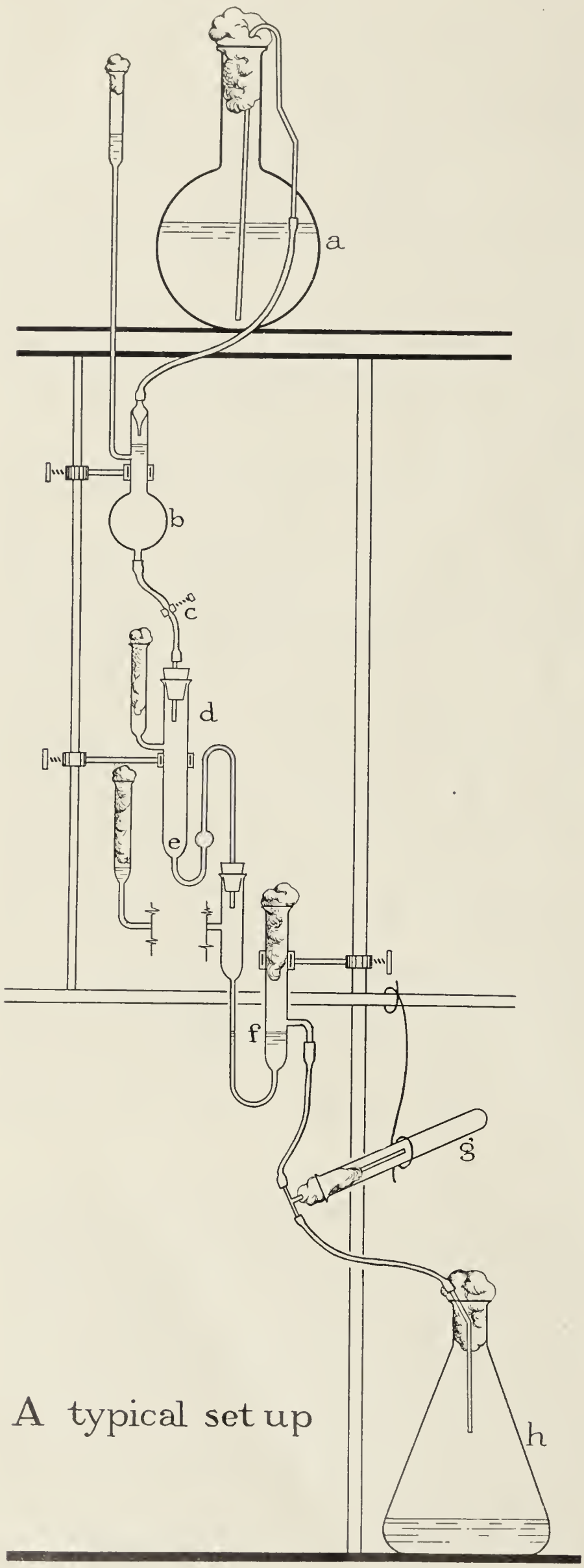
That it is possible to restore lost bacterial pathogenicity *in vitro* has been reported by some investigators. Thus, Rogers [1] claimed that non-virulent streptococci became virulent when grown on fresh rabbit serum. Von Lingelsheim [2] confirmed this result, but was unable to obtain as great a degree of restitution as Rogers. Cotoni,[3] using a gelatin peptone medium without body fluids, increased the virulence of certain strains of pneumococci. More recently, Wadsworth and Kirkbride [4] studied the effect of different media and intervals of transfer on the virulence of pneumococci. These investigators found it possible to maintain the virulence of this microorganism for a long period on ordinary or serum broth by making 8-hour transfers. They also report a restitution of virulence in certain strains of avirulent pneumococci by utilization of the 8-hour interval for reinoculation. The restitution was, however, slight,—not over a 10 per cent increase in virulence.

Bacteria have been quite exhaustively studied with reference to rate and type of growth as influenced by the various constituents of the medium, as well as its initial and final H-ion concentration. The bibliography is very voluminous on these subjects and no attempt will be made to give detailed references. Historical development with reference to growth has been well exhibited in Graham-Smith's excellent paper on "The Behavior of Bacteria in Fluid Cultures as Indicated by Daily Estimates of the Number of Living Organisms." [5] Clark [6] has completely covered the bibliography in regard to H-ion concentration. The results of all the investigations on the rate of growth of bacteria may be summarized as follows: (1) Inoculation of media is followed by a lag period which depends upon type of media and number of organisms in the inoculum (30 minutes to 6 hours); (2) a phase of growth acceleration or logarithmic multiplication (6 to 12 hours); (3) a stationary phase; (4) finally, a phase of logarithmic decrease. This cycle is accompanied generally by a change in the H-ion concentration of the media, the amount of change in great part depending upon the available carbohydrate which may be oxidized into an acid.

The facts presented in the above paragraphs, that the virulence of most bacteria becomes lower under ordinary conditions of cultivation *in vitro;* that restitution of this bacterial activity has been accomplished with any degree of success only by animal passage,—the increase by growth *in vitro* suggesting only a possibility of enhancing the virulence of a microorganism outside the animal body,—along with the realization of the undoubted importance of virulence of the microorganism as a factor in the spread of infectious diseases, stimulated this investigation. A method of approach which to us seemed logical was the study of the bacteria during the logarithmic increase phase, the assumption being that alteration in bacterial activity could be made with greater rapidity during this period of young growth than at any other period in the life of the microorganism. The difficulty immediately met with was making the transfers at such short intervals of time. To obviate this difficulty, the development of a mechanical method for making transfers seemed necessary. Accordingly, steps were taken to set up an automatic transferring apparatus. The development of such a device seemed rather simple in thought, but it was only after a number of trials that we succeeded with one which, although not ideal, gave interesting promise.

METHOD

The diagram here inserted represents the apparatus installed and is quite self-explanatory. The medium in a syphon flask (a) is connected to a trap (b) by means of rubber tubing. The capillary (d) through which the medium passes is small, to aid in controlling the flow of medium into syphon (e) by the clamp (c). Syphon (e) is connected to the growth receptacle (b) by means of a rubber stopper. The overflow tube of the growth receptacle (b) is of large enough bore (4 mm.) so that the outlet flow will be more rapid than the inlet flow. The large part of the apparatus is made from 15 mm. tubing and all other parts, with the exception of outlet (b), of 3 mm. tubing. Syphon (e) is made large enough to hold



A typical set up

about 15 c.c. of medium; the growth receptacle 7 c.c., or about half the capacity of the syphon (e). The outlet is connected by rubber tubing to a waste jar (h). A collecting tube (g) is inserted in the tubing which leads to the waste jar.

In actual operation, all apparatus, either separately or completely assembled, having been sterilized in the autoclave, the clamp (c) is opened and the rate of drops regulated so that the syphon (e) is filled in a desired length of time. The rate of flow having been registered and the regularity and time of syphoning determined, just after the receptacle is flushed out, inoculation is made by removing the plug from the growth receptacle (b). The usual procedure of transfer technique is used,—always making a rather heavy inoculation, about 1 c.c. of a 6 to 8-hour culture. Very little trouble has been experienced by contaminations; those that did occur were traced to cotton plugs which were too short or wet and to imperfect sterilization of rubber tubing. Some samples of tubing contain a very resistant sporebearing bacillus and sterilization can be made only by autoclaving at 18 to 20 pounds pressure for 25 minutes. The best grade of red antimony rubber is employed.

Experiments

As a test for the method here described, six experiments, each with different bacteria, were carried out with one kind of medium at a constant H-ion concentration. The medium was ordinary meat extract containing 0.02 per cent Liebig's meat extract, 0.5 per cent sodium chloride and 1 per cent Witte's peptone, titrated to pH 7.8. Medium was made in 3-liter lots and sterilized in the autoclave at 18 pounds pressure for 30 minutes. This length of time was found necessary to insure sterility of such large quantities of liquid.

Although it was found possible to obtain good growth at 1, 2, 4 and 8-hour periods of flushing in the case of all microorganisms studied, we shall confine this preliminary report to the 2-hour period.

Staphylococcus aureus.—The strain used was one isolated at autopsy from a case of staphylococcus septicemia. It was an intense pigment former on blood agar and its numerical virulence for mice was .001 c.c. of an 18-hour culture. After a run of three weeks, in all 240 transfers, 1 c.c. of an 18-hour broth culture had no effect on a mouse; the culture displayed very little pigment on a blood agar plate. Morphologically, it had all the appearance of a staphylococcus. However, the culture tended to be granular in its growth and settle to the bottom of the culture tube.

B. coli communis.—B. coli communis was an old stock strain without virulence for mice, biologically typical. After some 400 transfers, the only difference noted was the change in its morphology. At the beginning of the experiment the bacilli were rather short and plump, while

at the end there existed a preponderance of long "threads." However, the rods were Gram-negative and growth on the common sugars gave typical fermentation reactions. The culture was still avirulent.

B. typhosus.—In like manner these results were obtained with B. typhosus; the long "thread" form becoming especially abundant with this species of bacterium. As an avirulent stock culture it remained avirulent for mice; the culture maintained its typical sugar fermenting characteristics, but changed in its agglutinability; that is, the original stock organism agglutinated to a dilution with one specimen of typhoid immune serum to 1-20,000. At the end of the experiment, in testing the original culture in parallel with the culture after growing at the 2-hour period, it was found that the former agglutinated as it had before in a dilution of about 1-20,000, while the latter agglutinated in a dilution of 1-400,000 of the immune serum.

Streptococcus hemolyticus.—The strain of this organism used had been repeatedly passed through rabbits and had a numerical virulence for mice at the time of inoculation into the automatic device of 0.00001 c.c. of an 18-hour culture. After 500 flushings at 2-hour intervals, the strain had completely lost its virulence for mice and also its hemolytic activity as tested against rabbit's blood cells. The sugar reactions remained unaltered.

B. lactis aërogenes.—This organism was a strain which had been isolated at autopsy from a patient dying of pneumonia. It possessed great virulence for rabbits, cats and mice (as reported elsewhere),[7] one organism being sufficient to cause the death of a mouse in 24 hours. The morphology of this bacterium changed much less during the time represented by 324 2-hour flushings of the apparatus than the organisms mentioned above. However, quite a number of long forms appeared in the culture, and the capsule, so very large in the original condition, became markedly smaller. Fermentation reactions remained the same. The virulence for mice had decreased considerably: whereas at the beginning of the experiment one bacillus, as determined by the plating method, caused the death of a mouse in 24 hours when injected into the peritoneal cavity, 10,000 bacilli were necessary to produce the same result after the culture had grown under the conditions of this experiment. It is noteworthy that this strain of B. lactis aërogenes maintained its pathogenicity for a longer period than the strains of Staphylococcus aureus, Streptococcus hemolyticus or, as will be seen below, the pneumococcus.

Pneumococcus.—The strain used was a Type I pneumococcus of high virulence. An exact titration was not done, but 0.000001 c.c. of an 8-hour culture proved fatal for a mouse in 48 hours. At the end of a 300 transfer period, the organism had become greatly altered. It no longer grew turbidly, but in a granular fashion, like a hemolytic streptococcus; it had lost its specific agglutinative character; only an occasional organism was encapsulated, as determined by several methods for demonstrating the presence of capsules; and it had become absolutely avirulent for mice, a decrease of virulence of approximately 1,000,000 times.

Discussion

The results obtained in the above experiments have since been amply confirmed and suggestions for possible explanations have presented themselves. The reasons for the alteration in morphology, agglutinability and virulence of the microorganisms here studied, under the conditions of the experiments described, must be left to future investigations. There is one constant condition for all organisms with which we experimented other than the medium worth mentioning, namely, the H-ion concentration. From time to time H-ion concentration of the fluid in the growth receptacle was estimated colorimetrically. The concentration of H-ions remained constant with all organisms, the pH varying only from 7.4 to 7.6 throughout all the experiments. It is interesting that, although the H-ion concentration remained practically constant and approximately that of the blood of an animal, yet the virulence of Streptococcus hemolyticus, Staphylococcus aureus, B. lactis ærogenes and the pneumococcus decreased in marked degree. For these microorganisms we may infer that mere multiplication of the bacteria at the pH of the blood of an animal during its phase of logarithmic increase does not assure maintenance, much less increase of virulence. However, we do not assume that acid formation under ordinary methods of cultivation may be void of any action destructive to the virulence of a microorganism; only the converse of this is emphasized by these experiments.

It is not our intention to present at this time a definition of virulence. It would seem that the general concept is correct that virulence is a characteristic attribute or function of a microorganism enabling it to produce disease or death of the host: the numerical relationship for infection being a variable; the growth of the organism in or upon animal tissue an essential. Our experiments have supplied additional evidence in support of the postulate that virulence of a microorganism implies its ability to grow in or on animal tissue; that is, in the decrease of virulence of the organisms studied above, the bacteria lost, not their power of multiplication or existence, but their ability to live and grow in the animal body. As a corollary, although the number of experiments carried out was insufficient to draw any conclusion in regard to virulence and capsule formation, it is noteworthy that the decrease in the size of the capsule of B. lactis aërogenes and its loss on the pneumococcus in a state of decreased virulence of these organisms confirms Danysz's opinion [8] and that of many subsequent observers in regard to the

possible relationship of virulence and capsule formation of bacteria.

This paper is to be considered only in the light of a preliminary report with a presentation of the method. The apparatus described, with some modifications, has been in use since 1917. The results since obtained are confirmatory and other reports of the work will appear from time to time.

Summary and Results

1. An automatic device is described for the study of bacteria at various periods of transfers.

2. As a test for the method, six different microbic species—Streptococcus hemolyticus, Staphylococcus aureus, B. lactis aërogenes, B. coli communis, B. typhosus and the pneumococcus—were studied on a constant medium, meat extract broth, at a 2-hour period of transfer with the following results:

(a) A rather constant change of morphology was observed in all six species after a long period of transfers.

(b) Fermentation reaction of typical sugars remained constant as did the Gram stain for all organisms.

(c) Agglutinability of B. typhosus and the pneumococcus changed. The former became more agglutinable and the latter lost its specificity.

(d) The virulence of Streptococcus hemolyticus, Staphylococcus aureus, B. lactis aërogenes and the pneumococcus was markedly decreased; accompanying the decrease in virulence of B. lactis aërogenes, the capsule decreased in size and in the case of the pneumococcus entirely disappeared, as determined by several methods for capsule staining.

3. The method described presents the possibility of a systematic study on the influence of different conditions and a variation in food supply furnished the microorganism during the various periods of the growth curve.

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THE INCREASING SIGNIFICANCE OF PERMEABILITY-PROBLEMS FOR THE BIOLOGICAL AND MEDICAL SCIENCES

By H. J. Hamburger, Sc.D., M.D., LL.D., F.R.S.,
Professor of Physiology in the University of Groningen (Holland)

(The Charles E. Dohme Memorial Lectureship. First Course, Oct. 10, 11, 12, 1922)

Lecture III

1. *The reason why the glomerular membrane is normally impermeable to glucose.*
   (*a*). Size of the glucose-molecule.
   Configuration.
   Isomeric and stereoisomeric sugars.
   Quantitative differentiation of sugars by the glomerular membrane.
   (*b*). Scheme of Clowes.
2. *Remarks on diabetes.*
3. *The surface layer of the cell in permeability problems.*
4. *Excitation of living cells and permeability.*
5. *Summary.*

1. *The reason why the glomerular membrane is normally impermeable to glucose.*

In the second lecture it was shown that the glomerular membrane of the frog's kidneys retains the physiological amount of glucose if the perfusion liquid is a suitable one, namely, a Ringer's solution having the proper Ca-ion concentration. This fact is remarkable indeed, when we come to think that glucose is a crystalloid substance, and that other crystalloids such as sodium chloride, sulfate, phosphate and other salts pass through. To what must this peculiar and at the same time advantageous behavior be ascribed? I say advantageous behavior, for would it not be inexpedient, if the highly important carbohydrates, indispensable for muscular activity, were to leave the body unutilized?

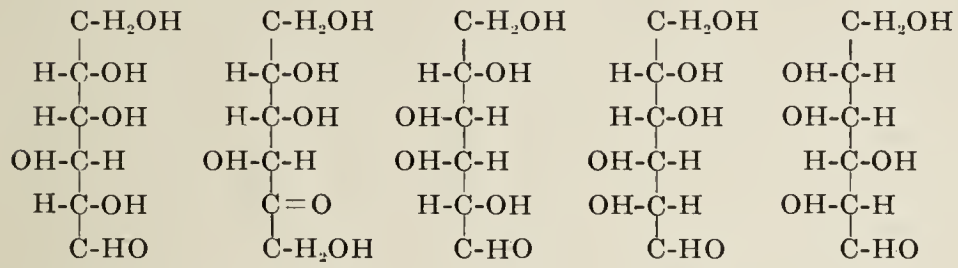
(*a*). Size of the glucose-molecule.
Configuration.
Isomeric and stereoisomeric sugars.
Quantitative differentiation of sugars by the glomerular membrane.

In the first place, we might imagine that the molecule of the monosaccharide glucose ($C_6H_{12}O_6$) is so large that its passage through the glomerular membrane would be hindered. At first we thought that if this notion were correct, disaccharides like sucrose (saccharose), maltose and

lactose, which have a still larger molecule ($C_{12}H_{22}O_{11}$), would certainly be retained as well. The experiments proved, however, that the glomerular epithelium is permeable to the three above-mentioned disaccharides to a large degree. It is even *perfectly* permeable to raffinose, which has a still larger molecule ($C_{18}H_{36}O_{18}$). Thus the size of the molecule could not be the determinative factor.

If then the retention of glucose could not be ascribed to the size of its molecule, there was the probability that a characteristic structure or configuration of the glucose molecule had something to do with it. For this reason numerous sugars which are either isomeric or stereo-isomeric with glucose were experimented with.

For the sake of convenience I give the formulæ of a few of these sugars:



And what proved to be the case? That fructose was allowed to pass through completely, likewise mannose, while only part of the galactose was retained. My audience will not fail to notice how slight is the difference in the configuration of these five sugars, especially between the *d*-glucose and its stereo-isomers galactose and mannose.

Glucose, therefore, occupies a very peculiar place amongst the isomeric monosaccharides with regard to the glomerular membrane; or, to express it differently, the glomerular epithelium has a power to distinguish glucose from other sugars in a way which suggests the relation of sugars to ferments. It is an established fact that a particular ferment can break up a certain sugar and that others cannot. Sugar and ferment fit each other, as Emil Fischer expressed it, like a lock and key. If we apply this to our case, we would say that the key (glucose) does not fit the lock (glomerular membrane) and that the other sugars which have so far been experimented with do fit and pass through.

The power of the glomerular (or tubular) epithelium * to distinguish *d*-glucose from other sugars goes so far—and this is not without importance from a clinical point of view—that when *d*-glucose (dextrose) and lævulose are dissolved in the perfusion liquid, the dextrose is again retained by the glomerular membrane, but the lævulose passes through. From this it is evident that the power of retention for dextrose is quantitatively not altered. The two sugars are simply separated from each other as through a filter. What is true for a mixture of dextrose and lævulose also applies to a mixture of glucose and lactose. The lactose passes completely into the urine and the glucose is retained by the glomerular epithelium to the same extent as when there is no lactose present.

It is deserving of attention that these investigations form a new illustration of the law of isomerism and stereo-isomerism, but here it is not of a chemical, but of a physiological nature—as they occur in the domain of permeability.

We may repeat, therefore, that it is the configuration of the glucose molecule that imparts to this substance the peculiarity that causes it to be retained by the glomerular epithelium.

In order to discover to what group of atoms in the glucose molecule its retention is due, we have by long continued investigation examined several other stereo-isomeric sugars, amongst others several pentoses such as xylose, arabinose, etc., and some hexoses like galactose.

Amongst these there are some that passed through the glomerular membrane, others that could do this only partially. Of those sugars that showed partial retention, we have examined more exactly *d*-galactose and *l*-xylose. Of these two sugars the *d*-galactose has special importance, as it takes part in the constitution of the central nervous system. This partial retention, which always amounted to about 50 per cent in the case of *d*-galactose and to 25 per cent in the case of *l*-xylose, seemed to me very remarkable. For upon closer examination it appears to agree very little with the idea of permeability that a sugar should be only partially retained, because a filter either holds back a substance, or allows it to pass through unconditionally. A mean between these two extremes can hardly be conceived when we have to do with only a simple substance and not with a mixture.

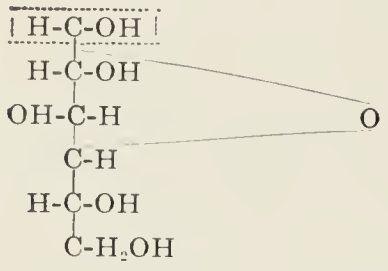
At this stage we recalled to mind that, according to the investigations by the French chemist Tanret, there are different known varieties of galactose and xylose, which in a watery solution hold each other in equilibrium. This is equally valid for other sugars, to which point I shall return later. Thus I concluded that the reason for the partial retention must lie in the fact that there are two modifications of *d*-galactose when it is dissolved in water, namely the α- and the β-variety. The α-variety is retained by the glomerular epithelium and the β-variety is allowed to pass through. The same holds good for *l*-xylose. And that such a distinct separation by the glomerular membrane of two sugars is possible, we have already learned from the mixtures of glucose and lævulose and of glucose and lactose. We remember that in both cases glucose was retained quantitatively, whereas the lævulose

* There is some controversy about the question whether it is the glomerular membrane that retains the sugar, or the epithelium of the tubuli, which owing to the suitableness of the perfusion liquid prevents reabsorption of the sugar leaving the glomerular membrane (A. N. Richards). It is only a question of a geographical nature.

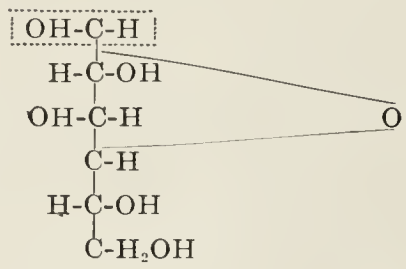
and lactose, by permeating the glomerular membrane *in toto,* passed into the urine.

It is obvious from the formulæ of both these α- and β-modifications that they differ only in the attachment of the H and OH groups to the asymmetrical carbon atom.

It is indeed remarkable that the behavior of the living membrane is dependent in such a striking way on such a small difference in the structure of two almost identically equal sugars. We write down the formulæ here a little differently:

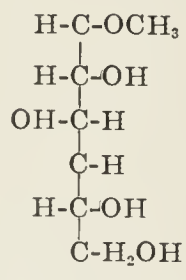


α-galactose, held back.

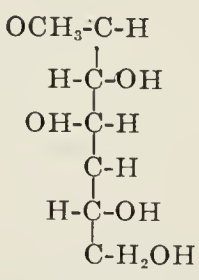


β-galactose, not held back.

Whether the α-form is held back and not the β-form, or the reverse, has not been established with such a degree of certainty as fully to satisfy me. The difficulties we encounter in such investigations are due to the fact that these substances do not remain pure. The dissolved α-form soon passes partly into the β-form and the same thing happens with the β-form in solution. The two forms hold each other in equilibrium. In other words: the galactose is subject to mutarotation, so that in an aqueous solution there exists only for an extremely short moment a pure α- or β-form. Pure forms can be obtained, however, by replacing the H of HCOH by CH_3, *i.e.,* by changing it into a glucoside. Then we get α-methylgalactoside or β-methylgalactoside:



α-methylgalactoside.



β-methylgalactoside.

The galactosides do not show any mutarotation; they do not change therefore into one another when in a dissolved condition. The α-form appears to be held back completely. The same must hold for the xylose-varieties.

I now have to consider the question how we are to interpret the mechanism of permeability.

There are two theories which attempt to explain the process of permeability; the one is based on the polarised condition of the surface of the cell and this may be called the electrical theory; the other is the sieve-theory.

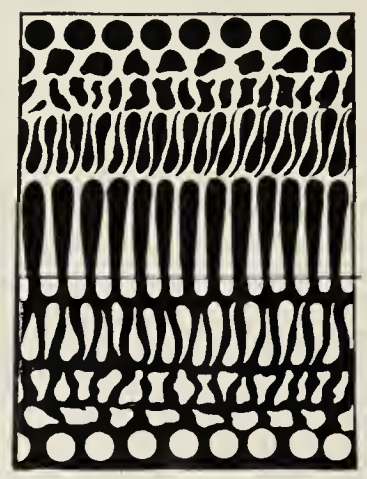
The first is embodied to a certain extent in *Girard's* scheme, and bears especially on ionic permeability. We have previously mentioned in Lecture II Girard's scheme in connection with the influence of acidity or alkalinity on the movement of the ions.

(*b*). Scheme of Clowes.

The second theory, *M. Traube's* sieve-theory, is illustrated by the representation of Clowes. It would take me too far afield to consider both these representations in detail. Allow me to say the following with regard to Clowes' theory:

The work of Clowes originated in an observation made by Bancroft (1913). He noticed that a mixture of oil in water can be emulsified permanently in two ways; the one emulsion consists of oil-drops suspended in a continuous watery phase as in cream; the other consists of drops of water suspended in a continuous phase of oil as in butter. Clowes converted an emulsion of the first kind, that is, of oil in water, into an emulsion of the second kind, that is, of water in oil, by simply shaking an emulsion of oil in water with a solution of calcium chloride. The reverse took place when shaking with sodium hydroxide. It is clear that when this is applied to the surface layer of cells, one will obtain two different states of permeability on the addition of Na or of Ca: Ca causes an oily canal-system and Na a watery canal-system. From this it follows that Ca will create an impermeability to watery substances. In other words: in the matter of permeability Na and Ca are antagonistic to each other. The following somewhat modified scheme of Clowes illustrates the experiment very well.

CLOWES' SCHEME (Modified)



Transformation of emulsion of *oil* in water to emulsion of *water* in oil by means of a slight increase of the concentration of Ca··ions.

Imitation of the scheme of Clowes.—In this scheme the oil is black and the water is white. Where no Ca is present in the surrounding fluid, you see oil globules in a continuous phase of water. Thus in the higher part of the scheme there does exist permeability to solutes; in other words, the canals allow any watery solution to pass. After the addition of 0.005% Ca, there is still a possibility for watery solutes to pass into the globules.

This state is changed by adding more Ca-ions (part of the scheme below the line). Then watery solutions are no longer allowed to pass. However, only molecules of certain shapes can pass. *These shapes change on the addition of more Ca,* for the Ca-ions dominate them, because the boundaries vary with the surface tension.

In applying these statements to the sugars, we can imagine that *d*-glucose is not allowed to pass through, whereas lævulose and stereo-isomeric sugars like mannose (and the β-variety of galactose and xylose) are able to do so.

You see, I have drawn a line. What is sketched above the line occurs in physiological circumstances; thus, *e.g.,* blood corpuscles treated with cobra-poison are not permeable to water. This corresponds with the part of the scheme below the line.

If this is applied to our experiments on the kidney, it is clear that the glomerular membrane becomes impermeable to glucose with an efficient concentration of Ca-ions in the perfusion liquid.

It is evident that the representation of the American author is strongly supported by our experiments on the stereo-isomeric sugars. We have seen that pores are left existing between the oil-drops, and it is obvious that these pores, because they are subject to the surface tension at the boundary, assume varying shapes. Now pores of a definite shape will allow sugars of a definite configuration to pass through, but they will hold back sugars of another configuration. I wish to recall to mind again our observations that when we allow a mixture of glucose and lævulose to flow through the kidneys, the lævulose passes through completely, but the glucose is held back quantitatively. One might be inclined to explain this phenomenon by the aid of differences in viscosity or surface tension of the sugars. But we have found by experiments that these physical constants are the same for lævulose and for glucose. How, therefore, can one explain this separation of glucose from lævulose otherwise than by assuming that the shape of the ultra-microscopic pores in the sieve play a decisive part? You will remember that from a mixture of glucose and lactose only lactose passes through, and also that only one of the two modifications of galactose (α- and β-galactose) is allowed to pass through. In this way, then, our experiments are supporting evidence of the significance of Clowes' theory and consequently *vice versa;* the mechanism of the permeability to glucose and other substances has become clearer. In this respect I may allude also to the remarkable studies of your countrymen—Harkins and Langmuir.

In laying stress on the fact that the shape of the pores depends upon the concentration of the Ca-ions, I have to revert for a moment to the fact observed in the experiments on blood corpuscles, that the presence of a definite amount of Ca-ions in the fluid is necessary. When there is a superabundance of Ca-ions in the fluid, the effect on permeability is the same as if there were too few Ca-ions. Now it may be remarked that one comes across an analogue of such a contrast in the recent important experiments of Neuschlosz. This investigator found that an emulsion of lecithin in sodium chlorid solution possesses a surface tension, which depends on the amount of Ca that is added to it. The Ca must be present in a fairly constant concentration to be able to diminish the surface tension to a definite degree. *Too much or too little Ca has the same effect.* Further, Neuschlosz found that K-ions work in an antagonistic way on the Ca-ions, with regard to the surface tension of lecithin emulsion. It will be remembered that the surface layer of the cells also contains lecithin to a considerable amount. What the reason is that the surface-tension of lecithin-emulsions is so sensitive to Ca-ions in the way I just mentioned, we will not discuss here. In the meantime, biologists have reason enough to be satisfied whenever they observe analogues in a vital phenomenon which they can produce experimentally in inanimate mixtures. It remains for the physical chemist to give an adequate explanation of the phenomenon.

Before leaving the stereo-isomeric sugars, I may be allowed to add a few remarks.

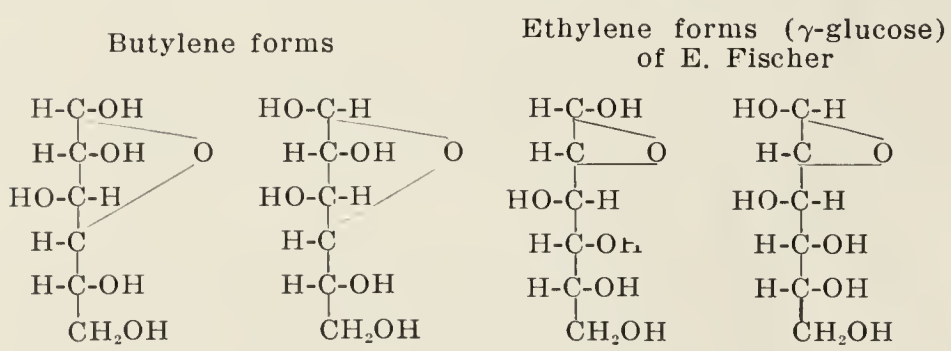
In the first place I will point out that our investigations on the subject suggest a number of questions that may have an important bearing on the study of diabetes.

2. *Remarks on diabetes.*

First, it may be asked: is the substance that in diabetes passes through the kidney really the ordinary *d*-glucose only or is it one of its stereo-isomers? This question is impressed upon us the more, since we have become acquainted with the researches very recently published by Csaki of Vienna. He made use of a method, applied in our laboratory by *van Creveld,* by which blood, caught in a paraffin-coated tube, is immediately centrifuged. You will remember that the blood-plasma is then shown to contain all of the blood-sugar. *Csaki* was able to confirm this result. However, when he experimented with blood from diabetic patients, part of the sugar was found to be present also in the blood corpuscles.

Two possibilities will here be thought of: (1) the blood of diabetics contains substances which make the erythrocytes permeable to sugar. (2) The sugar, at least part of it, is not the ordinary *d*-glucose, but a stereo-isomer. which can pass through the normal blood-corpuscles of man. The latter possibility seems most probable, since almost simultaneously two Japanese investigators, Kotaki and Okagawa, in connection with our experiments on the kidneys, have also shown a difference in permeability of blood corpuscles to stereo-isomeric substances, namely, to the three forms of hydroxyphenyl-lactic acid. They discovered that only the lævo-rotatory form was taken up by the blood corpuscles.

There are still other experiments showing the importance of the stereo-isomerism of sugars in connection with the problem of diabetes. Hewitt and Pryde started out to determine the relative velocity of absorption of glucose and fructose in the small intestine. In so doing they met with an unexpected phenomenon. They found that a solution of *d*-glucose, introduced into the duodenum of the rabbit, very soon shows a decrease of its rotatory power. In one case the rotation became almost negative. This phenomenon must be due to the formation of a negative or, at any rate, a weakly positive rotary form of a stereo-isomeric glucose, produced by contact with the living intestinal epithelium. That we really have to deal with the formation of a stereo-isomeric sugar of a low rotatory power becomes apparent from the fact that the fluid, after removal from the gut, soon regains the original rotatory power of ordinary *d*-glucose, namely, 52.5°. I shall not enter upon a further description of these experiments; I will only show you that, apart from the ordinary α- and β-glucose, there still exists another glucose, which is not the well known butylene form, but the ethylene form. Emil Fischer has given the name of γ-glucose to this form. Here are the formulæ:



(1) α-glucose (2) β-glucose (3) α-glucose (4) β-glucose

Now this γ-form has an extremely small rotatory power, much below 19°. It will be asked: what is the meaning of this conversion in the intestine? Does this substance remain in the circulation and if so, does it keep up there an equilibrium leading, as in the case of diabetes, to a shifting in favor of this stereo-isomeric sugar, to which the renal epithelium and also blood corpuscles are quite permeable? Is, furthermore, the trace of glucose, always present in normal urine, such a stereo-isomeric substance? These are questions that at present cannot be answered. However this may be, this much is certain, that the name "blood-sugar" is no simple concept. Probably we have to do with an equilibrium between the normal *d*-glucose and its stereo-isomers. The latter pass through the kidney. So it would be clear that also in normal men there is always a trace of sugar in the urine. Experiments on the *humor aquæus,* carried out with van Creveld, have also given results that make the presence of stereo-isomeric sugars in the blood probable. Our investigations on the different behavior of the renal epithelium towards stereo-isomeric sugars, which we would not have been able to perform without the efficient salt solution, make this question, with diabetes, one of paramount importance.

3. *The surface layer of the cell in permeability problems.*

So far we have dealt with the influence of the *medium* on permeability, but we have said nothing or scarcely anything concerning the significance of the structure of the *cell surface* which, of course, is not the same in different cells. Is it necessary to make this clear? It must be evident to every one that, in connection with difference of function, the surface layer of the intestinal epithelial cells, for instance, must possess a chemical and morphological structure which differs from that of the blood corpuscles and other cells. From the very nature of the subject, the study of the influence of the chemical and morphological structure of the surface layer of the cell on permeability is a much more difficult problem than that of the effect due to the surrounding medium. This for several reasons. In the first place, we have not the power to modify the chemical and morphological structure as we have to modify the surrounding fluid. Secondly, the cell surface and the fluid medium are difficult to separate, and even supposing they could be easily separated, the benefit therefrom would be small from a physical point of view, since permeability depends on a cooperation between the solid and fluid surface layer. This will soon become clear if we consider for a moment the cells of the salivary gland. Why does the substance formed in this gland go toward the lumen and not in the direction of the contractile tissue? No doubt because the boundary layer between the lymph and the salivary gland cell must have other properties of permeability than the boundary between the salivary gland and the saliva. A third reason, accounting for our deficient knowledge of the properties of the surface layer of the cell body, is the fact that there are so few varieties of cells that can be isolated without being damaged. It is the blood corpuscles again that are best, nay, almost exclusively, fitted to be studied for this purpose; yet here too we meet with difficulties. Must we consider the external layer as a part of the cell body or is it something derived from the plasma by absorption? A little later we shall go into this question more fully.

I believe we are all agreed that the surface layer of the erythrocytes, as they appear in the plasma or in the serum, consists of protein and lipoids. These lipoids are made up of a mixture of phosphatides, which have recently been so admirably analyzed by Levene, and of cholesterin. Now, this mixture has of late been thoroughly investigated by Dr. Brinkman and Miss Van Dam. These researches have led up to important results, both from a physiological and pathological point of view. They are also of significance for serology. A detailed discussion of these systematic investigations would take me too far; I can only give a brief account of

them. First, it has been shown that lecithin and cholesterin are substances of an entirely different physiological function; that they are antagonistic as regards permeability. The following simple experiment will make this clear. If blood corpuscles be washed with the ultra-filtrate of blood serum, *i.e.,* blood serum deprived of protein and thus consisting almost entirely of the serum salts, then the osmotic resistance of the blood corpuscles is increased. What does this mean? It means that the erythrocytes can now tolerate a weaker salt solution than before without being hæmolysed. Let me tell you, by the way, that instead of the ultra-filtrate, a suitable sodium-sulfate or sodium-phosphate solution may also be used, not however a common salt solution. In the last something entirely different happens. Thus, we find that blood corpuscles of defibrinated blood begin to lose coloring matter in a 1.5 per cent. sodium-sulfate solution, then, after being washed in an ultra-filtrate or in Ringer's solution, they will be able to tolerate not only a 1.5% Na-sulfate solution without losing any coloring matter, but even solutions of 1.4 and 1.3 per cent concentration. Why is this? Because lecithin has been washed off from the surface layer of the cell and, indeed, it can be demonstrated in the washing fluid by means of a delicate test, found by myself. That we have really to deal with a removal of lecithin by the washing process is corroborated by what we see when lecithin is added to the blood corpuscles. Then it is found they cannot stand a Na-sulfate solution even in the strength of 1.6 and 1.7 per cent. From this it may be inferred that lecithin lowers the resistance against diluted salt solutions, in other words, it weakens the osmotic resistance of the blood corpuscles. This can be shown not only by experiments *in vitro,* but also *in vivo.* If we administer to a rabbit lecithin intravenously for a few days, hæmolysis with subsequent hæmoglobinæmia will ensue. Furthermore, by a method of resistance determination, worked out in the Physiological Institute at Groningen, a powerful regeneration of young blood corpuscles is shown to be associated with this hæmoglobinæmia. It also appeared that a sufficient amount of lecithin in the food is indispensable for a normal resistance and a normal regeneration. When rabbits were made anæmic by bleeding, a regeneration of blood cells was scarcely noticeable provided that they were fed only on grass and beets, both of which contain little lecithin. But regeneration became normal as soon as food rich in lecithin, like oats, was added. The same statements have been made by Whipple and Hooper in their work on blood regeneration. Lecithin, therefore, stimulates the formation of erythrocytes. Further experiments have brought out the fact that the surface layer of the red blood-corpuscles contains but little lecithin at the moment of their birth in the bone marrow, and that they absorb it from the plasma as soon as they have found their way into the circulation. That this absorption really exists is shown by the fact that the corpuscles, after being washed in the ultra-filtrate by which their osmotic resistance is increased, regain their original lower resistance on being brought back again into the serum. There exists in this regard a quantitative difference between man and the rabbit, but into this problem I will not enter here. Nor shall I dwell on the possibility of differentiating primary and secondary anæmias by determining the resistance of washed and unwashed blood corpuscles.

Now researches of very recent date, performed in our laboratory by Bolt and Heeres, have further shown that the spleen is the organ in which the erythrocytes receive substances that activate the lecithin. If the blood is allowed to flow through the spleen, the erythrocytes coming from the splenic artery appear to have acquired a lower power of osmotic resistance. This is in accordance with the results of R. M. Pearce, described in his book on the spleen and anæmia. From a pathological point of view this is an important fact. It is well known that erythrocytes are continually destroyed in the liver and this destruction, it is evident, will be intensified if the blood corpuscles have become less resistant. From this intensified destruction anæmia results. Now, guided by these experiments, the osmotic resistance was determined, in the medical clinic of Groningen University, in a patient suffering from a profound anæmia. The resistance was found to be much lowered and this gave support to a proposed surgical measure—extirpation of the spleen, an operation performed before on empirical grounds. The resistance of the blood was thereby increased and the patient did well.

So much concerning the significance of lecithin. Before broaching the subject of cholesterin, I may be allowed the remark that, in serological work, washing of the blood corpuscles is not without its influence on hæmolysis.

Now as to cholesterin.

This substance is also an ever-present constituent of the surface layer of the cell; it is the antagonist of lecithin, with which it is mixed. It is difficult to separate cholesterin from the blood corpuscles by means of salt solution; with a cane sugar solution one succeeds better, as shown by the presence of cholesterin in the latter after washing corpuscles in it. We therefore found that an isotonic cane sugar solution in which rabbit's blood corpuscles are washed becomes red and, as far as we could ascertain, the same happens with blood corpuscles from other animals. It is due to a disturbance of the equilibrium between lecithin and cholesterin, which equilibrium was discovered in numerous researches of Bloor and his coworkers, so that lecithin, which, as we saw, decreases the resistance, is chiefly left behind. Both substances are present in a definite proportion and this constant relation must be regarded as of prime importance. Directly dependent on this fixed relation is the

resistance of the erythrocytes, the electrical isolation of the cell, the permeability of the surface layer to ions and its concentration of water. Dr. Brinkman and Miss Van Dam succeeded in finding a method by which the hæmolytic activity of lecithin in 3 c.c. of human blood could be determined.

Just a few words concerning the influence of these substances on the electrical isolation of these cells. It is well known that the erythrocytes from women's blood settle sooner than those derived from the human male. This seems to be due to the greater amount of cholesterin in women's blood, corroborated by the fact that blood of pregnant women settles still quicker than that of those not gravid. It also appears that the biconcavity is a phenomenon caused by cholesterin. Years ago I observed that the blood corpuscles lose in NaCl solution their biconcavity and take on the globe shape. On studying the reason for this occurrence, which was the starting point for the researches on lecithin and cholesterin in our laboratory, it appeared that if the globe-shaped corpuscles are brought back into a saline solution containing 0.1 per cent cholesterin, the erythrocytes again assume their biconcave form.

We now leave the study of the surface layer of the red blood corpuscles and its theoretical and practical significance, to conclude by considering the relation between excitation of living cells and permeability.

4. *Excitation of living cells and permeability.*

In the introduction to my lectures, I mentioned, as an example of the significance of permeability for vital processes, transmission of stimuli by means of chemical substances produced on stimulation. When, for instance, the vagus is stimulated, substances are set free which are capable of stimulating again the peripherical ramifications of the vagus in another heart. On excitation of the sympathetic, something similar may be observed.

From the very nature of the matter, I have not entered into the many problems that are urged upon us in this chemical field; for instance, I have left untouched the mechanism by which stimulation of nerves leads up to liberation of those substances in the heart. For the moment one can do little more than advance hypotheses, and, for further progress, they are needed, however risky they may seem. Better a problematic hypothesis than a passive submission to fact. The latter would mean scientific death and we do not wish to fall again into the error of the old vitalism. Thus, let us picture that on stimulation of a nerve there occurs a displacement of potential towards the junction between nerve and muscle, by which change of potential surface tension is modified, which modified tension results in a change of permeability. In view of this modified permeability, substances accumulated in the muscle cell may now leave the muscle. Hence there is liberation of substances also on excitation by stimuli that are not exclusively electrical in nature; they may be mechanical also. That the substances which have been set free induce a new stimulation has been experimentally demonstrated; I only recall to your mind the experiments by Demoor and ours on the heart-stomach preparation. Change of surface-tension, brought on exclusively by nervous stimulation, might also be thought of.

Anyone in doubt as to the velocity with which a decrease of surface tension is transmitted need only be reminded of Lord Rawleigh's well known experiment. A piece of camphor is put on the surface of water; the camphor is seen to make restless movements, which may be considered as a phenomenon of surface tension. Now put a finger into the water at some distance from the camphor and suddenly the movements stop.

But I shall not lose myself in hypotheses with regard to the mechanism by which nerve stimuli are transmitted and with regard to their point of attack. Ever since the birth of the physiology of nerves and muscle, from the days therefore of Helmholtz and du Bois Reymond, these difficult problems have occupied the minds of scientific men. And any one desirous of knowing the modern conceptions and the interesting experiments pertaining thereto may consult the magnificent work by R. S. Lillie, to say nothing of the investigations by Nernst, Fletcher, MacDonald, A. V. Hill and others.

I would rather come back to the experimental evidence for the relation between stimulation and changes of permeability. And I will mention three examples. The first is taken from plant physiology. Excitation of Mimosa gives rise to electrical phenomena with a simultaneous exudation of elements of cell sap (Lucca).

The second example refers to investigations by Lillie on the larvæ of Arenicola Crystata. Here we have cells, colored by a yellow pigment that is soluble in water. If the cells are irritated, they contract and part of the pigment goes out of the cell; the stronger the stimulus, the greater the amount of pigment that escapes. This excitation may be effected only by bringing the larvæ into an unbalanced solution, for instance, by transferring cells from sea-water into a pure sodium chloride-solution. On adding a definite amount of Ca-ions, excitation ceases and there is no more discharge of coloring matter. Here a disturbance of the normal equilibrium in the surrounding medium affects the cell in such a manner that an increased permeability results. This case is analogous to what J. Loeb demonstrated years ago with reference to frog's muscle, which also contracted when immersed in a pure sodium chloride solution. The contractions ceased when to the salt solution other ions, as Ca-ions, for example, were added. It is evident, therefore, that the surface changes may give rise to contractions.

Noyons of Louvain has recently drawn attention to a method by which it is possible to determine changes in the surface layer of the cell which till then could not be directly measured. He made use of an apparatus in-

vented by a Dutch physicist Dr. Moll, the so-called extinctiometer. If light be thrown on a suspension of blood corpuscles in a sodium chloride solution, the corpuscles reflect both light and heat rays. He detects these reflected heat rays by means of a thermopile, to which a galvanometer is connected. If now he adds to the NaCl solution, which, as you know, has a lyotropic (softening) action on the blood corpuscles, a little calcium chloride, then the amount of reflected heat is changed, as can be read on the galvanometer.

So much for the experiments of R. Lillie on the larvæ of Arenicola.

A third example of the relation between stimulation and change of permeability does not relate to lower cells, but is concerned with muscles. Of late a thorough investigation into the origin of increased permeability has been instituted by Embden and his pupils of Frankfort. Embden's results were briefly the following: The fresh gastronomic muscle of a frog, directly after preparation, gives off in Ringer's fluid, in which it is immersed, a certain amount of PO_4. If the muscle is not stimulated, the discharge of PO_4 by the muscle cells gradually diminishes and finally ceases completely. After stimulation of the muscle, a typical increase of PO_4 results. The stronger the excitation, the greater the PO_4 secretion. If slight fatigue ensues, the PO_4 discharge soon ceases after excitation. If the fatigue has become excessive, so that the muscle loses its irritability, the muscle continues to lose a great deal of PO_4 during this period of non-irritability. Concomitant with the reappearance of irritability is a decrease of PO_4 discharge, which at last becomes minimal. The permeability to substances containing nitrogen also increases during excitation. It is, therefore, a well established fact that here excitation and increased permeability are associated phenomena. The question again rises: which of the two is the primary process? Into this I shall not enter here.

These examples may suffice. It would, no doubt, lie in the line of my discussion to broach the subject of narcosis. The modern theories of narcosis, as, for instance, expounded by Winterstein in his monograph, attribute to the phenomena of permeability an important rôle.

There is one thing I would ask you to pay attention to once more. In my introduction I remarked, with reference to the heart-stomach experiment, that irritability involves a cooperation between ions and organic substances. There are several examples that may be adduced in support of this conception. Adrenalin (epinephrin) has a sympathetic effect only when there is a definite concentration of Ca-ions; another proportion between K and Ca renders epinephrin inactive or causes it to have a vagus effect. Choline does not influence the heart without K-ions; digitalis has no action in the absence of Ca-ions. How they act together, we do not know. And so we see again that as we penetrate deeper into the processes of irritability, by chemical and physico-chemical methods, the question of the nexus between them brings up new questions again as soon as we discard the purely vital theory; and thus it goes on.

But time presses and I must come to an end. But before doing so, I wish to recapitulate what I have said and will attach to this summary a few remarks of a general nature.

5. *Summary.*

In studying the reason why the glomerular membrane is normally impermeable to free glucose, we put to ourselves the question, whether this fact is to be ascribed to the size of the molecule; but this could not be the case, seeing that molecules which were considerably larger passed through the membrane. We therefore came to the conclusion that the configuration of the molecule must be the cause. It was indeed found that sugars which were isomeric or stereo-isomeric with glucose pass through. This even happens to such an extent that these sugars can be separated quantitatively from glucose. If a mixture of glucose and lævulose be introduced into the perfusion liquid, only the lævulose passes through. These facts strongly support Clowes' representation, namely, that surfaces of cells and also here of the glomerular membrane behave like a sieve of which the ultramiscroscopic pores can be of different shapes, the shapes being dependent on the concentration of the calcium ions. How could the above-mentioned quantitative separation of glucose from its stereo-isomers and also the separation of the α-form and β-variety of galactose and of the two xyloses be otherwise explained?

These experiments introduce again a great number of questions which become important for the study of diabetes, especially in connection with the occurrence of stereo-isomeric sugars. I am thinking of the experiments of Hewitt and Pryde dealing with the change of α- and β-glucose into γ-glucose in the intestines, of the permeability of red blood corpuscles to only a part of optical antipodes, etc., etc. All these considerations furnish working hypotheses for the elucidation of diabetes-problems, still so puzzling at this moment.

After our remarks concerning diabetes, we showed that the permeability of a cell does not depend exclusively on the surrounding fluid, but also on the surface layer of the cell itself. Yet we had to point out that it is very difficult to know how much is due to the cell surface and how much to the effect of the surrounding fluid, which by absorption is attached to the surface layer of the cell. In this connection we dealt with the significance of lecithin and cholesterin that coat the surface of the blood corpuscles and discussed their meaning from a physiological, pathological and serological point of view. And finally, the relation between excitation of living cells and permeability was put forward and we saw that excitability and changes of permeability are associated phe-

nomena, not only with regard to ions, but with regard to organic substances as well. Here too it was evident again how great a significance problems of permeability have for an approach to questions that until a short time ago still were considered as of a purely vital nature.

A consideration of the latter leads us to ask ourselves, how far we shall succeed in "understanding" life!

On one thing we are agreed: rigid vitalism has collapsed. No one doubts any more that physical and chemical phenomena determine to a considerable extent the phenomena of life, yet there is no agreement as to whether something else, something unknowable that is specific to life, may not play a rôle besides. Those who hold this view we called *neo-vitalists* in opposition to others named *mechanists* who, in this regard, have less decided opinions, but who do not exclude the possibility that the gap between the two may be bridged over some day. And there are unquestionably some grounds for the latter view. A century ago, every one was convinced that not a single substance produced by the living organism could ever be made in the laboratory; but in 1828 Wöhler succeeded in making urea artificially. A number of other similar substances followed, as for example tartaric acid, derived from the vegetable cell. However, it was soon discovered that the artificially prepared tartaric acid lacked a property that belongs to the natural acid; it had no effect on light. Such a tartaric acid, it was believed, could be produced only by means of vital force. The adherents of the vital force theory rejoiced, and were not silenced when Jungfleisch succeeded in manufacturing malic acid which in every respect is identical with the natural product. Still other phenomena formerly thought to belong to the living cell are at present looked at in a different light. I remind you of the conversion of sugar by the living yeast cell. Following Pasteur, this conversion was generally held to be a vital phenomenon proper to these lower organisms; now we know that it is dependent on the action of a ferment residing in the yeast cell, which may be squeezed out of the cell, and we also know that a similar action may be effected by other ferments, even by those belonging to inorganic nature. Do not these examples urge us to beware against putting up the barrier—ignorabimus? The neo-vitalist does not seem to be affected strongly by this consideration, by this warning. Today he sees that a phenomenon of life, formerly held to be entirely unaccountable and regarded by him therefore as a specific revelation of vital force, can be satisfactorily explained by physico-chemical facts. Yet soon this explanation brings forth new questions. Again these lead to the discovery of new facts that seem to defy any physical or chemical elucidation and at once we see the neo-vitalist ready to hold up these facts as a vindication of his view. Certainly, he says, chemical and physical facts no doubt play a rôle, but if we probe deeper into the matter, they do not seem to account completely for the phenomenon. But behold, after some time an experiment removes the obstacle again. And again our insight has deepened, and new riddles crop up afresh. And thus it goes on forever and ever. "On avance toujours, on n'arrive jamais." But does not exactly the same thing happen when we study so-called dead nature? How does the mechanist regard the ever newly arising difficulties? Taught by experience how often physics and chemistry give an obvious explanation where, a long time before, no solution seemed to be possible, he has become more prudent, is less soon discouraged, may be, is also of a less mystic disposition. He is full of hope and confidence that, as so often has happened before, physics and chemistry again will shed their light, and as long as this is absent, he patiently awaits and contents himself with saying: Ignoramus, we do not know yet. Fortunately neo-vitalists and mechanists stand in their laboratories shoulder to shoulder and in their work-gowns they cannot be differentiated. Moreover, is the gulf between the two groups of naturalists at bottom so very wide? I speak of naturalists, ignoring every dogmatism, which so often dominates the mind. The boundary line between life and death is becoming more difficult to draw. Herbert Spencer in his well known work needed two chapters and a half to define life. We hear already of inorganic life and we ponder when we think of radium and the ever continuing evolution of this metal into other elements. Yet, though the gap between the two may not be entirely bridged over, both groups of research men may be one in their loftiest and highest feelings and in their mutual reverence for the greatness of Nature and consciousness of their own insignificance. They may take to heart Goethe's words: "The highest happiness of the thinking man is to have discovered the discoverable and patiently to admire the inscrutable."

NOTES ON NEW BOOKS

The Oxford Index of Therapeutics. Edited by Victor E. Sorapure. Cloth, $12.00. (*London, Henry Frowde; and Hodder and Stoughton, 1921.*)

A review of a work of this sort is always an ungrateful task, because at most one can hardly be more enthusiastic than about a good dictionary. A brief encyclopedic index of therapeutics must necessarily be incomplete, and through its incompleteness must at times convey direct misinformation, or at least a false sense of security, to the reader. Within the limits of possibility, however, the present work seems altogether creditable, and provided the reader does not seek for more than he may reasonably expect to find, the book should have considerable use for quick reference along therapeutic lines. A. L. B.

Clinical Tuberculosis. By FRANCIS M. POTTENGER. 2nd Edition. (*St. Louis, C. V. Mosby, 1922.*)

The work consists of two volumes of 1290 pages of text and 180 excellent plates, charts, and illustrations. It is well edited and the charts especially are well chosen and instructive. The chapters are well arranged and are conveniently punctuated with expressive sub-titles facilitating ready reference.

It is quite possible that many readers will be somewhat dismayed at the maze of reiteration to be contended with before they are able to get at the meat of the matter. The purpose of this repetition, as explained by the author, is well taken, from his own viewpoint; nevertheless, it imposes voluminous reading on the busy practitioner for whose instruction the book was originally prepared. There are several chapters which could be condensed to a third or even one half of their volume and yet lose none of their force for any one who has an elementary knowledge of physiology and pathology. The chapters on the digestive system and on fever in tuberculosis are typical of this group.

The unabridged discussion of the nervous system reveals unusual information on the subject by a student-clinician; but after all it is mostly of contributory importance and is very apt to stray into the field of speculation.

The chapter on "relationship between the physician and patient" is especially timely and should be thoroughly learned by those treating invalids for a disease so chronic as is tuberculosis. It takes the patient out of the catagory of "case" and makes him a co-worker with his doctor.

The chapters on "open air" and "climate" are conservative. Ignorance and tradition of the past have thrown a hazy atmosphere about climate in its relation to tuberculosis. Climate is one of those complex and flexible therapeutic agencies handed down from the ancients to meet the unknown demands of consumption. It was depended upon by Galen to dry up the corrupt secretions which he thought were causing the illness. With the modern and scientific analysis of consumption has come a more critical analysis of climate and no one locality in any part of the world has been found to contain even a small group of the components once included in the all-inclusive term of climate, without having a share of injurious elements. In other words, there is no ideal climate. It is refreshing to read from the pen of one who lives in one of the "favored climates." "It (climate) should always be made secondary to the intelligent supervision of the patient." And....."It can be seen that while climate is a complex resulting from many factors, the good or bad influence of the climate depends on the degree to which a man can adapt himself to the particular elements present and maintain a physiological equilirbium." With as much force could *financial* be substituted for *physiological.* We of the "unfavored climates" have seen irreparable damage done, morale destroyed, and valuable time and money lost in the vain search for that "particular climate wherein the individual can best obtain and maintain that necessary physiological balance."

The chapter on tuberculin is an energetic defense of an extensive use of that agent in the face of its rapidly declining popularity. There are those who will fail to see the practical difference between tuberculin as a "specific" and tuberculin as having "specific action." The selective power of tuberculin for small or beginning tubercle as stated on page 349, volume 2, is very desirable but unfortunately only theoretical. "General reaction" may be an "accident" but certainly must be dealt with as a positive condition.

The author's criticism of the wide-spread therapeutic nihilism in the treatment of clinical tuberculosis is thoroughly justifiable. The disdain of some clinicians for medicines in this connection smacks of ignorance or lack of interest, but conversely, polypharmacy bears the same ear-mark. The author falls short of his opportunity here to create a sane and standard medical therapy of tuberculosis by a lack of positiveness in the recommendation of remedies which have proven unquestionably their preeminence in their respective indications. He could have stated more forcibly "Acute pleurisy is *best* treated by strapping the chest." "Heroin is the most effective cough remedy" and "Creosote is the drug *par excellence* in tuberculosis." To advocate the use of Pituitrin and condemn that of horse-serum in the treatment of stubborn pulmonary hæmorrhage is founded on personal experience in the realm of empirical therapeutics.

The chapters dealing with diagnosis are good. The author dwells on the minor importance heretofore placed on Inspection and Palpation. His elaboration of these two procedures is a distinct contribution to the science of diagnosis of disease of the lungs. Whether his clear discussion of reflex muscle spasms, their causes, detection and interpretation, will place palpation as the second step of importance in the physical examination of the chest is yet to be seen; but that it has been grossly neglected by most of us is not to be denied. To be able to say emphatically that "the thickened pleura is accompanied by a peculiar doughy or edematous feel to the overlying muscles" comes only after years of cultivation of palpation.

The author takes a sane view of the value of X-ray in the diagnosis of pulmonary tuberculosis. His summary of the discussion should exert a distinct check upon those who have been making the diagnosis from the chest plate and requiring the signs and symptoms to dove-tail in accordingly. He says with truth: "It requires as much experience and skill to interpret properly a plate as are required to interpret physical findings."

We should have been glad to have Dr. Pottenger's views on the relation of war gases to tuberculosis and on the influence of some of the industrial factors that are receiving current attention.

On the whole the work exhibits a wide knowledge of foreign and domestic literature and a rich personal experience with clinical tuberculosis.

There are certain parts that show a distinctly personal coloring which can be produced only by the scholar and the scientist after some 15 or 20 years' devotion to study. In the field of science when the scholar approaches debatable ground or "no man's land," he naturally relies on his own experience and advances it as dogma. For such cases, for the time being, the opinion of one earnest investigator is as acceptable as that of another, for only time and conscientious investigation can be relied upon to establish the truth.

M. F. S.

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